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A study of cuticular sense organs on the legs of *Gerris remigis* Say (Heteroptera: Gerridae), with special reference to a chemosensitive basiconic sensillum and its putative rôle in mating behaviour

by

Kristopher Paul Fennie

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

Department of Entomology

EDMONTON, ALBERTA

Spring 1987

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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A study of cuticular sense organs on the legs of *Gerris remigis* Say (Heteroptera: Gerridae), with special reference to a chemosensitive basiconic sensillum and its putative rôle in mating behaviour submitted by Kristopher Paul Fennie in partial fulfilment of the requirements for the degree of Master of Science.

B. K. Mitchell
.....
Supervisor

.....

.....

Date April 15, 1987

Abstract

A cataloguing of cuticular structures on legs of *Gerris remigis* is presented. Hair-layers and sense organs are discussed. A description of previously undescribed basiconic sensilla on the trochanters and femora of mesothoracic and metathoracic legs of *Gerris remigis* males is given.

A survey of 25 species in seven genera reveals that these basiconic sensilla are on the mesothoracic trochanters and femora of males and females of *Limnoporus dissortis*, *Limnoporus notabilis*, and *Limnoporus rufoscutellatus*. The distribution of these basiconic sensilla suggest they were independently derived twice, once in these three species and once in *Gerris remigis*. *Gerris najas*, the sister species of *Gerris remigis*, does not have the basiconic sensilla.

Histology of the legs of *Gerris remigis* reveal that each basiconic sensillum is multi-innervated with 11 to 15 neurones. Multi-innervation and apparent small pores on the sensillar surface suggest these are multi-porous chemosensitive organs.

Mating behaviour in *Gerris remigis* is described. Behavioural experiments designed to elucidate the function of these basiconic sensilla were conducted. It is suggested that they are chemosensitive and detect a contact pheromone produced by the female. Evidence in favour of this is: (i) sensilla are present only on adult males; (ii) a behavioural act of leg-rubbing is performed by the male on female's abdomen during mating; (iii) the ability to successfully mate in darkness. Evidence not in favour of a pheromone-mediated mating system is: (i) mating occurs after blocking receptors of these sensilla; (ii) experiments where models are treated with extract of slurryed females and presented to males show no receptiveness to males concerning mating.

The basiconic sensilla are probably chemosensitive. They function in mating behaviour, but are part of a larger mating system involving visual, vibrational, and chemical cues. All three cues are important, but not all three cues need be present for successful mating to occur.

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Table of Contents

Chapter	Page
Abstract	iv
Acknowledgements	v
List of Tables	ix
List of Figures	x
1. Introduction	1
1.1 Ancestral form and adaptation	1
1.1.1 Ancestral form	1
1.1.2 Leg adaptations	2
1.1.3 Hair-Layer adaptation	3
1.2 Locomotion	5
1.3 Sensory aspects	7
1.4 Behaviour	9
1.4.1 Prey capture	9
1.4.2 Mating behaviour	10
1.5 Definition of thesis problem	12
1.6 *Gerris remigis* life history	13
1.7 Literature Cited	18
2. A Catalogue of Cuticular Structures on the Legs of *Gerris remigis*	22
2.1 Introduction	22
2.2 Materials and Methods	24
2.3 Results	25
2.4 Discussion	29
2.5 Literature Cited	47

3. A Survey of Basiconic Pegs in Gerridae 51

3.1 Introduction 51

3.2 Materials and Methods 52

3.3 Results 54

3.4 Discussion 54

3.5 Conclusion 58

3.6 Literature Cited 65

4. Histological Aspects of the Trochanter and Femur of *Gerris remigis* 67

4.1 Introduction 67

4.2 Materials and Methods 67

4.3 Results and Discussion 70

4.3.1 General Histology 70

4.3.2 Epidermal cell-layer 70

4.3.3 Mechanosensitive Structures 71

4.3.4 Basiconic Sensilla 72

4.4 Conclusion 73

4.5 Literature Cited 81

5. A Study of Mating Behaviour in *Gerris remigis* 82

5.1 Introduction 82

5.2 Materials and Methods 83

5.3 Results and Discussion 86

5.4 Conclusion 94

5.5 Literature Cited 101

6. Conclusion 102

List of Tables

Table		Page
5.1.	Data of mating sequence in *Gerris remigis*.	98
5.2.	Data of mating sequence in *Gerris remigis* with antennaectomized males.	99
5.3.	Data of mating sequence in *Gerris remigis* with blocked receptors.	100

List of Figures

Figure		Page
1.1.	Five postural phases in locomotion.	17
2.1.	Three Leg-pairs	34
2.2.	Acetabula and Coxa.	34
2.3.	Extrinsic leg muscles	34
2.4.	Macro-hair layer on mesothoracic femur	36
2.5.	Macro-hair and micro-hair layers.	36
2.6.	Joint sculpture of femoral-tibial joint of mesothoracic leg.	36
2.7.	Close up of joint sculpture.	36
2.8.	Micrograph showing rough cuticle and pit pores.	36
2.9.	Close up of pores	36
2.10.	Close up of pit pore.	38
2.11.	Micrograph showing striations on a macro-hair.	38
2.12.	Mesothoracic tarsus	38
2.13.	Close up of L-shaped hairs showing inclination.	38
2.14.	Macro-hair layer including setae on metathoracic femur	38
2.15.	Close up of setae.	38
2.16.	Prothoracic tibia and tarsus	40
2.17.	Close up of grooming comb.	40
2.18.	Knobs or pores at base of grooming comb.	40
2.19.	Location of small campaniform sensillum	40
2.20.	Small campaniform sensillum with "tail".	40
2.21.	Elongated campaniform sensillum set in cuticular swelling.	40
2.22.	Arrangement of three elongated campaniform sensilla.	42
2.23.	Location of three elongated campaniform sensilla.	42

2.24. Spindle-shaped campaniform sensilla. 42
2.25. Location of the spindle-shaped campaniform sensilla. 42
2.26. Sensory hair plate. 42
2.27. Location of sensory hair plate. 42
2.28. Trichobothria on femur and trochanter. 44
2.29. Trichobothrium base. 44
2.30. Metathoracic pretarsus showing three tarsal hairs. 44
2.31. Basiconic sensilla on metathoracic femur and trochanter. 44
2.32. Lateral view of basiconic sensillum. 44
2.33. Dorsal aspect of basiconic sensillum. 44
2.34. Surface of basiconic sensillum. 46
2.35. Subcuticular glandular material. 46
2.36. Glandular material with ducts running to cuticular surface. 46
3.1. Distribution of basiconic sensilla on mesothoracic trochanter of a male. 60
3.2. Lateral aspect of sensillum. 60
3.3. Dorsal aspect of sensillum showing base. 60
3.4. Pores on the surface of a sensillum. 60
3.5. Distribution of basiconic sensilla on mesothoracic femur of a female. 60
3.6. Lateral aspect of sensillum. 60
3.7. Distribution of basiconic sensilla on mesothoracic femur of *Limnoporus dissortis* female. 62
3.8. Lateral aspect of sensillum on mesothoracic femur of *Limnoporus dissortis* female. 62
3.9. Distribution of basiconic sensilla on mesothoracic femur of *Limnoporus rufoscutellatus* male. 62
3.10. Lateral aspect of sensillum on mesothoracic femur of *Limnoporus rufoscutellatus* male. 62

3.11. Pores on surface of sensillum of *Limnoporus dissortis* male.....62
3.12. Cladogram of relationships between subfamilies of Gerridae according to Andersen (1982).....64
3.13. Cladogram of relationships between genera in the subfamily Gerrinae according to Calabrese (1980).....64
4.1. Cross-section at joint.....76
4.2. Longitudinal-section at joint.....76
4.3. Cuticle and epidermal cell-layer.....76
4.4. Gland cell in epidermal cell-layer.....76
4.5. Cuticle showing pores.....76
4.6. SEM photomicrograph of these cuticular pores.....76
4.7. Socketed hair and campaniform sensillum.....78
4.8. Basiconic sensillum and receptor cavity.....78
4.9. Dome and receptor cavity of a campaniform sensillum.....78
4.10. Basiconic sensillum and enlarged epidermal cell-layer.....78
4.11. Suggested structure of the basiconic sensillum sense organ.....80
5.1. Ethogram of mating sequence in *Gerris remigis*.....97

1. Introduction

Water-striders (Gerridae; Heteroptera) demonstrate grace and beauty by their soft velvety appearance and their unrestrained fluid movement across the water surface — as if not to make a sound or leave a trace upon the water. Moreover, it is intriguing that, inspite of their apparant idyllic way of life, they survive in an environment that can be harsh. It is their combined robustness and gracefulness that make them remarkable; it is their unique adaptations that allow them to have these qualities, that makes them particularly interesting to study.

Gerrids belong to the infraorder Gerromorpha or semi-aquatic bugs. There are over 12,000 known species of Gerromorpha; 85 per cent of known species belong to the families Gerridae and Veliidae (the two most-derived families of the infraorder) (Andersen 1982). All Gerromorpha live in damp areas usually with free-standing water. Members of Gerridae and Veliidae are the most successful in adapting to the water surface.

1.1 Ancestral form and adaptation

1.1.1 Ancestral form

According to Andersen (1982), the ancestral gerromorph lived in damp locations where it was accessible to water. Because of this, the gerromorph entered new and distinct adaptive zones on the water surface, or to be more exact, the air-water interface. There are three major zones in which gerromorphs live: (i) transitional zone between terrestrial and aquatic, where there is little free water, rather, water film and drops (e.g., decaying vegetation, gravel); (ii) small water patches, vegetation covered with water (e.g., tree holes, crab burrows); (iii) open water surface (fast-flowing or slow-flowing streams, stagnant ponds)(Andersen 1982).

The third zone characterises best the habitat for members of Gerridae and Veliidae. They live in

habitats from temporary puddles to the open ocean (such as *Halobates*). While there are Coleoptera, Diptera, Collembolla, Acari, and Araneae that live on the water surface, the Gerridae and Vellidae (as well as other gerromorphs) have developed unique adaptations including major morphological and behavioural changes, which especially suit them for their environment. The changes in Gerridae — and to a lesser extent Veliidae — will be discussed here.

1.1.2 Leg adaptations

A major structural deviation from the ancestral heteropteran found in these two groups concerns legs. The mesothoracic and metathoracic legs are separated posteriorly from the prothoracic legs, giving the impression of being almost inserted on the abdomen. The mesothoracic and metathoracic acetabula, and coxae, are directed caudad. The mesothoracic coxa is enlarged latero-ventrally (this modification accomodates powerful extrinsic muscles necessary for the thrust motion in rowing) (Andersen 1982; Bowden 1978b).

The prothoracic leg is the shortest of the three leg-pairs. The function of this leg-pair is to aid in balance during rest and locomotion, and in prey-capturing and mating. The prothoracic leg is modified for grasping (including having an enlarged femur and tibia). In the case of prey-capture, the water-strider orients, ~~grabs the prey~~ with its prothoracic legs, and impales the prey with its mouthparts while securing it with the prothoracic legs.

The mesothoracic leg is the longest of the three leg-pair; the tarsus is especially elongated. (This is different than in the groundplan of land-dwelling Heteroptera where the metathoracic leg is longer.) The rôle of the mesothoracic leg is mainly in locomotion; it provides the impetus of the thrust stroke. It is also involved in orientation, steering, and in mating and communication (to be discussed later).

The metathoracic leg is similar in form to the mesothoracic leg. The tarsus is not as elongate as

the mesothoracic tarsus. The metathoracic leg functions in balance, and to a certain degree, in locomotion. Again, it has a rôle in orientation, communication, and, possibly mating.

1.1.3 Hair-Layer adaptation

Another major adaptation of Gerridae (and other semi-aquatic bugs) is the hydrophobic hair-layers that cover the body including the legs. These hair-layers are a necessity for prevention of wetting of cuticle and overall protection against moisture. The velvety sheen appearance of these insects is due to the hair-layer structure. Andersen (1976, 1977, 1982) gives a detailed description of structure and function of the hair-layers. There are two hair-layers: (i) macro-hair layer; and (ii) micro-hair layer. (These terms are used *sensu* Andersen (1977), however, it should be pointed out that the micro-hair layer is actually composed of micro-trichia, which are non-articulated, non-innervated cuticular outgrowths. Andersen's terms are used here in order to minimize confusion.)

The macro-hair layer consists of various types of long, pliable, socketed (and, thus, probably innervated) hairs. In *Gerris lacustris*, the majority of hairs are 40 – 60 μm in length, tapering from a width of about 2 μm to a point (Andersen 1977). These measurements appear to approximate those of other members of Gerridae. The hairs are, in general, fluted or ridged. In addition to these hairs are trichobothria that can extend in length to 100 – 150 μm (see chapter 2); and more stout hairs or setae with a base width of 15 – 20 μm, the flutes are more pronounced on these larger hairs (see chapter 2). Save for trichobothria (which are at 80 – 90 degree angles), the macro-hairs are inclined at angles of 20 degrees on distal leg parts to 50 degrees on the body. Inclination of these hairs is integral in achieving effective hydrophobic action.

The density of the macro-hair layer also plays an important rôle in maintaining dry cuticle. However, the hair density varies widely among individuals and species. Andersen (1977) estimates hair densities of 3000 – 5000 per square millimetre on the lower part of the pleuron in

Gerris lacustris, whereas in a species of *Halobates* one macro-hair type occurs in densities of 8000 – 12,000 per square millimetre. On the head, prothoracic lobe, and more dorsal parts of the insect are hair densities less than those mentioned aboved.

The micro-hair layer is composed of non-articulated, non-innervated cuticular outgrowths that densely cover the body. In *Gerris remigis*, for example, the hairs are 4 – 10 μm in length and about 0 · 6 μm wide; spacing is about 1 – 7 μm (Andersen 1977). Micro-hair densities are greater than macro-hair densities. On the mesosternum of *Gerris lacustris*, for example, one finds densities of up to 5 – 8 x 10^5 per square millimetre (Andersen 1977). The distribution of micro-hairs is less variable than that of macro-hairs; legs tend to have few, if any, micro-hairs, and the cuticle is smooth or slightly tuberculate (see chapter 2).

Hair shape and structure are highly variable. The most common micro-hair tapers distally, but remains rounded at the end. Projection of most hairs is 90 degrees to the cuticular surface. Some micro-hairs bend foward distally, such hairs usually appear in groups comprising a field of bent micro-hairs. Some hairs are modified distally into a clubbed, beveled, or flattened shape; this is especially noticeable in species that are highly specialised, for example, members of the genus *Halobates* (an open ocean living water-strider).

Andersen (1976, 1982) discusses the rôle of hair-layers in maintaining position on the air-water interface and in locomotion. The surface film is deformed by the weight of the water-strider. The water-strider does not break through the surface film because there is a resisting force that pushes the area of the legs in contact with the water surface upward. This resultant force is dependent on the area of the leg in contact with the water surface; the surface tension of the water; and the angle of deformation of the water surface film, which Andersen refers to as the contact angle. The greater the hydrofuge properties, the greater the angle of deformation will be (always greater than 90°). (For the mathematical expression of this and a more detailed explanation, the reader is referred to Andersen (1976).)

The hair-layers are very hydrofuge. This is due to the shape of the hairs and area of contact with the water surface, and possibly due to oils or waxes covering the hairs. The hair-layers, with their hydrofuge properties, have a high contact angle, and it is precisely this that allows them protection from wetting. It is of prime importance that the hair-layers are maintained in proper position and clean; this is why water-striders are often seen grooming.

1.2 Locomotion

Hydrofuge properties of hair-layers of these insects play an integral part in locomotion, although this may, at first, seem contradictory. While it is stressed that water-striders rest on the air-water interface, there is one moment (in locomotion) where it breaks through the water surface film; this perforation is made by the (pre-apically inserted) tarsal claws. The insect is able to protract the tarsal claws, break the water surface film, because the angle of deformation is less than 90 degrees, and thereby gain resistance which it may use in locomotion — and movement in general (Andersen 1976). When the claws are extended, and because of the extra force due to the mechanics of leg movement, the meniscus is increased *viz.* in depth (to approximately twice that of a resting *Velia* (Veliidae)) (Andersen 1976). Darnhofer-Demar (1969) used this phenomenon in investigations of locomotion by observing the shadow of the miniscus on the substrate. From this, Darnhofer-Demar concluded that waves were generated by leg movements as a way to increase resistance by applying the leg to the back slope of the wave. This allows an increase in power and ultimately speed in locomotion (Andersen 1976). During locomotion, the distance and speed at which a water-strider is able to move is dependent on the power of the propulsive forces and the drag (created by the legs on the water-surface)(Andersen 1976). The drag on a water-strider is small due to a high contact angle as well as the ability of the insect to lift itself off the surface. The actual mechanics of locomotion of water-striders — including dynamics, kinematics, functional anatomy, motor control, and behaviour — have been extensively worked out (principally) by Andersen (1976,1982); Bowdan (1976, 1978a, 1978b); Darnhofer-Demar (1968, 1969, 1973, 1977); and

Murphey (1971a, 1971b).

Members of the families Veliidae and Gerridae have evolved a mode of locomotion (termed rowing), at the expense of walking (Bowdan 1978a). Although the water-strider is able to walk on land, the action is uncoordinated. Bowdan (1978a) suggests that proprioceptive input is of little importance in walking because of the unpredictability of order, number, or combinations of leg protraction. Yet, there exists weak coordination (intersegmental as well as intrasegmental coordination)(Bowdan 1978a).

In contrast, rowing is a very well-coordinated means of locomotion in which the mesothoracic legs are the major propulsive organ, while the prothoracic and metathoracic legs function as a tetrapod support (Andersen 1982; Darnhofer-Demar 1968).

A complete stroke cycle can be divided into five phases (Andersen 1976): (i) ***stationary***, in which all three leg-pairs are resting on the air-water interface; the force of the mesothoracic leg-pair is not as great as that of the other two leg-pairs. The distal end of the prothoracic tarsus is in contact while the whole of the tibia and tarsus of the metathoracic leg is in contact with the air-water interface. (see figure 1.1a) (ii) ***beginning thrust***, in which the mesothoracic legs synchronously push in a caudad direction. Meanwhile the weight of the insect is moved to the metathoracic legs and the prothoracic legs are lifted off of the surface. (see figure 1.1b) (iii) ***thrust***, is a continuation of phase ii where the mesothoracic legs fully extend and complete a stroke and both the prothoracic and metathoracic legs are off the surface. (see figure 1.1c) (iv) ***beginning recovery***, this is the gliding phase where the insect is not touching the water surface. (see figure 1.1d) (v) ***recovery***, the insect makes contact with the surface, first with its prothoracic legs and then metathoracic legs. The mesothoracic legs are recovering, so they do not touch the surface, rather, they are moved foward to a position so that another complete stroke may take place (see figure 1.1e) (Dornhofer-Demar 1969; Andersen 1976, 1982). Steering and turning are controlled by unequal thrust of the mesothoracic leg-pair, and a rudder-like action of the metathoracic leg ipsilateral to the direction of the turn (Andersen

1976, 1982).

Andersen (1976) determined the duration of certain phases. The thrust phase is 20 - 30 milliseconds and the recovery phase is variable, lasting up to 60 milliseconds in *Gerris lacustris*. It appears that the water-strider takes no more than ten strokes per second, each stroke propelling it up to 1 · 5 centimetres, in addition to the gliding phase which is approximately three to seven times this distance.

It should be mentioned that there exists two types of stroking in the Gerridae. The first is 'gliding' (Capanigro & Eriksen 1976) in which there is low frequency rowing and minimal change in prothoracic and metathoracic legs. The second is 'leaping' (Capanigro & Eriksen 1976) or 'jumping' (Andersen 1982) which is an escape mechanism in which the water-strider jumps up because of the power of the thrust, to a height of one centimetre in some cases; the whole animal is in the air and the thrust phase speed can reach 100 - 130 centimetres per second (Andersen 1982).

Simultaneous rowing of mesothoracic legs in the water-strider is an intriguing adaptation to life on the water surface. It is a faster, more efficient means of locomotion — taking advantage of the physical properties of the water-surface tension and hydrofuge properties of the insect's hair layers — compared to that of primitive tripod type of locomotion found in most Heteroptera (including the lesser-derived semi-aquatic bugs). This method of locomotion contributes greatly to the success of this insect.

1.3 Sensory aspects

Members of the family Gerridae have well developed sensory systems, especially on the legs. The prothoracic, mesothoracic, and metathoracic ganglia are condensed into a single mass in the prothoracic region, with major nerve trunks leading to peripheral organs, such as legs. A wide array of mechanosensitive organs are found on the legs; these include three types of

campaniform sensilla , trichoid sensilla, innervated hairs, (i.e., Type I or cuticular mechanosensitive organs); and Type II sensory organs (non-cuticular mechanonsensitive organs) such as chordotonal organs. It is understandable that these insects have an extensive number of sense organs, since legs play a vital rôle in the Gerridae.

Members of Veliidae and Gerridae are able to perceive water-surface waves as signals to detect potential predators and prey as well as determine conspecifics (Andersen 1982). In some species males and or females signal by creating surface waves. Presence or absence of these signals can indicate sex, territory, spacing, and mating call (Andersen 1982; Walker 1983; Wilcox 1972, 1979, 1980a; Wilcox & Kashincky 1980).

Evolution of this type of sensory system in Veliidae and Gerridae was possible because of their exploitation of open water, where there is little vegetation, debris, and other possible blockers of surface waves (Andersen 1982). In open water, there are also many waves — or background noise — which are of no value to the insect; they have, therefore, evolved surface wave perception of certain frequencies and amplitudes, thus, minimizing and filtering noise (Andersen 1982). For example, Rensing (1961) found *Gerris lacustris* responsive to ripples less than 4μm and greater over a large frequency range of 20 - 500 Hz, with peak response in the range of 150 - 200 Hz. Wilcox (1979) found *Gerris remigis* responsive to two different frequency ranges and ripples of 1μm amplitude or greater. The first frequency range was 20 - 50 Hz — signals produced by struggling prey; the second frequency range was 85 - 90 Hz — produced by conspecific males as a sex-recognition behaviour (Wilcox termed the high frequency signals 'HF' signals).

The actual sense organs involved in detection of surface waves, despite considerable research effort, are unknown. Lawry (1973) hypothesised that trichobothria located on the femora, tibiae (labelled trochanters in Lawry (1973)), and tarsi of *Gerris remigis* detect surface signals when in contact with water. Moreover, Lawry found a reduced response to vibrations in animals with ablated trichobothria. It is known that invertebrates do use trichobothria to detect

air vibrations (Schuh 1975). Murphey (1971a, 1971b), in a thorough study of motor control and sensory aspects of orientation to prey in *Gerris remigis*, found that localisation of prey occurs by several turns, the degree of turning depending on distance from prey (as well as direction). The mesothoracic leg-pair and the metathoracic leg ipsilateral to the prey are the legs most involved in a turn. And, the phase of firing of motor neurones of these legs is, again, dependent on distance and direction of prey (Murphey 1971a). Through ablation experiments, Murphey suggested that omni-directional mechanoreceptors are located in the tibial-tarsal joint, in the distal part of the tibia, or in the first tarsal subsegment. Localisation of the source of waves occurs by determining which sense organ — and necessarily leg — is nearest to that source; from this, the insect is able to orient by variable turns (Murphey 1971b). From Murphey's work, it would appear that, indeed, since ablation of the tibial-tarsal joint produced a reduced response to vibrations, there is a sense organ (most likely a Type II sense organ) present in the tibial-tarsal joint region. No electrophysiological or histological studies have been carried out to confirm this hypothesis.

1.4 Behaviour

1.4.1 Prey capture

Gerrids are 'waiting predators' in that they rely on the water surface to either transmit signals from a potential prey, caused by struggling, or to physically bring prey within reach (Andersen 1982). Freshwater gerrids feed mostly on terrrestrial insects that have become trapped on the water, or on emerging adults (which have aquatic immatures). While gerrids show a clear preference for prey that is alive, they will, when starving, feed on dead animals. In a study of five species, Jamieson & Scudder (1979) found that a gerrid will usually not respond to prey if it must turn more than 100 degrees. The degree to which a gerrid will turn and the distance it will travel to capture prey varies with hunger.

Gerrids rely on visual cues in locating and orienting towards prey, especially at close range (Jamieson & Scudder 1979). But, as mentioned above, gerrids are able to localise struggling prey by surface waves generated by the prey (Liche 1936; Murphey 1971a; Wilcox 1980 Andersen 1982). According to Wilcox (1979), *Gerris remigis* responds to waves of a frequency of 20 – 50 Hz, this being the frequency of waves generated by struggling prey. Orientation to the prey is by small turns until the prey is directly in front of the water-strider (Murphey 1971b). At this point the gerrid moves rapidly in a straight line towards the prey. With its raptorial prothoracic legs, the gerrid grabs the prey and impales the prey with its feeding stylets. The gerrid stays in this positon until satiation (Andersen 1982).

(Gerrids may also use surface waves, in conjuction with visual cues, as a means of detecting predators such as fish.)

1.4.2 Mating behaviour

An even more intriguing use of surface waves by gerrids involves mating behaviour. Many gerrids — possibly all — produce and detect signals through surface waves to distinguish potential mates, competitors, and oviposition sites. Systems vary with different species.

Wilcox has done considerable research concerning the rôle of signalling in mating behaviour. In *Rhagadotarsus kraepelini* (a pond-dwelling gerrid in Australia), Wilcox (1972, 1979) found that the signalling system is complex and well developed. A male produces signals for (i) pre-copulatory calling and courtship; (ii) copulation; (iii) post-copulation; (iv) individual spacing; (v) territoriality; and, (vi) oviposition site (Wilcox 1972, 1979). Both males and females are responsive to these signals, females being sensitive enough to discriminate between frequencies differing by 1 · 5 Hz (Wilcox 1972,1979). A male responds to territorial and spacing signals as well (Wilcox 1972,1979). The male frequently uses a stationary object, such as a stick or leaf, as a site from which to signal; the female, after copulation, uses this as an oviposition site (Wilcox 1972,1979). A female produces courtship signals only (Wilcox 1972,1979).

Gerris remigis has a simpler system; and, in this respect, probably better represents the Gerridae as a whole. Only the male signals, and only the male responds. The signal is the HF signal of 85 – 90 Hz. The male uses this signal when near a conspecific to discriminate sex. If the conspecific receiving the signal is male, he will signal in response; then, they will quickly separate, or, in some cases, fighting will ensue. If prior to signalling there is an attempt to mount, the male being mounted will commence HF signalling. If the conspecific is female, there is no response, and the male will usually attempt mounting (Wilcox 1979; Andersen 1982). While females show no overt response to HF signals, they may perceive them.

Signalling systems have been worked out in other species such as *Limnoporus dissortis*, *Limnoporus notabilis*, and *Limnoporus rufoscutellatus* (Spence & Wilcox 1986; Wilcox & Spence 1986; Vepsäläinen & Nummelin 1985). The systems closely resemble that of *Gerris remigis*. The male signals and only the male responds. There are, however, differences in behaviour (i.e., causes of signalling, types of responses).

It is not known how important or to what extent signalling is involved in mating behaviour. As in prey-capture, it appears that visual cues are important in mating behaviour. Visual cues are not necessary for mating to occur, however. (Wilcox 1979; Andersen 1982).

Chemical cues may also be involved in mating. There is some evidence that chemical cues may exist in some specices of water-striders (Spence personal communication). This will be discussed in greater detail in chapters 3 and 5. Moreover, the function of the metathoracic scent gland has not been determined. Among several hypotheses related to the function of this gland, it has been suggested that it functions in mating (e.g., producing a pheromone) (Andersen 1982). However, Wilcox (1972) found chemical cues to be unnecessary for mating behaviour since males responded to models in the presence of experimentally generated signals. This does not, however, mean that chemical cues do not exist, only that mating is possible without cues other than surface signals.

Mating behaviour (including territoriality, and male-male interactions) has been described in *Rhagadotarsus kraepelini* (Wilcox 1972), *Gerris najas* (Sattler 1957), *Limnoporus dissortis* and *Limnoporus notabilis* (Spence & Wilcox 1986; Wilcox & Spence 1986), and *Limnoporus rufoscutellatus* (Vepsäläinen & Nummelin 1985), among others. A detailed description of a mating sequence in *Gerris remigis* is presented in chapter 5.

1.5 Definition of thesis problem

Because gerrids live in a unique habitat — on the air-water interface — they have evolved many adaptations conducive to survival. Modifications and adaptations of the legs are especially interesting to study because of their essential and intimate association with the water-surface. Extreme modification can be seen; structurally, as in lengthening of legs, caudad positioning of acetabula, pre-apical claw insertion, hair layer; functionally, as in enlarged extrinsic muscles, jump and glide locomotory mechanism; behaviourally, as in escape mechanisms, locomotion, grooming, mating; and neuroethologically, as in prey detection, mating-signals, and motor control.

Concerning detection of surface waves, Murphey (1971b) determined that there is a lack of proper orientation after ablation of the tarsus and distal end of the tibia, and concluded that there must be a sense organ located in the tibial-tarsal region. However, no histological or electrophysiological work was carried out to confirm the presence of a Type II mechanosensitive organ in this region. Lawry (1973) suggests that trichobothria are involved in detection of surface waves. His work involved an SEM study and some histological work dealing with neurones of trichobothria. Complete histological studies and electrophysiological studies were not carried out.

In order to better understand possible mechanisms of surface-wave perception, three areas require investigation: (i) a thorough SEM study of cuticular sensory structures, especially in

the tibial-tarsal joint area; (ii) a serial sectioning of the legs to identify appropriate neurones, again, paying close attention to the tibial-tarsal joint area for Type II sense organs; (iii) electrophysiological studies of the sensitivity to surface signals of identified sense organs.

This thesis provides data on the first two areas for *Gerris remigis*, a locally available species, which is well studied and large. In the SEM investigation, I noticed a sexual dimorphism involving sense organs of the legs. The mesothoracic and metathoracic femora of males contain apparent chemosensitive organs. The sexual dimorphism suggested that these sensilla could be involved in surface-wave detection; yet, their basiconic shape and apparent pores suggested a chemosensitive function.

To my knowledge, these sensilla have not been previously described. It is not known what the distribution of these sensilla are, if they are present only in *Gerris remigis*, or if they are widely distributed but have not been described. Also, little has been done on mating behaviour in *Gerris remigis*, therefore, there is no indication that *Gerris remigis* mating behaviour has 'behavioural acts' consistent with a pheromone system. Thus, I included in my study a section on distribution of these sensilla in other gerrids, and a section on the structure in *Gerris remigis*. Chapter 2 is a catalogue of cuticular structures on the legs of *Gerris remigis*. Chapter 3 is a survey of presence of the new basiconic sensilla in various species, as well as a more detailed discussion of the sensillum type. Chapter 4 presents the histology of the basiconic sensilla, Chapter 5 describes a basic mating sequence for *Gerris remigis*, discussing possible behaviours consistent with a pheromone system. Chapter 6 is an attempt to pull the various aspects together into a coherent analysis of the rôle of sensory systems in legs of *Gerris remigis*.

1.6 *Gerris remigis* life history

I chose *Gerris remigis* as the species on which to focus my study. From a practical standpoint, it was chosen because of its large size, availability, and because it has been well studied.

Gerris remigis Say (Heteroptera; Gerridae) is a large brown water-strider that inhabits flowing water such as streams, and is widely distributed throughout North America, and even into Guatemala (Drake & Harris 1934). It is possible that *Gerris remigis* is actually a species complex. There are at least three taxa that are involved in this putative complex in USA and Canada (an eastern, a western, and a southern taxon) (Stonedahl & Lattin 1982). For more information regarding this, the reader is referred to Stonedahl & Lattin (1982) and Michel (1962).

The body size averages 12 · 5 – 15 · 5 millimetres in males and 13 · 8 – 16 · 6 millimetres in females (Stonedahl & Lattin 1982); it is also more robust, being wider in the thoracic region, as compared to other getrids. The female is slightly larger than the male. Apterous, brachypterous, and macropterous morphs are seen in *Gerris remigis*. However, this is not a consequence of seasonality, a particular individual will either develop wings or will not. There tends to be little variation within a population, generally a population will be predominately apterous, predominately brachypterous, or predominately macropterous with few, if any, of the other morphs. The apterous form is, by far, the most common (Andersen 1982).

Gerris remigis prefers flowing water, and is frequently found on small streams with little vegetation, save grass or sedges on and near the banks. It spends much time patrolling on open water, and, when in need of protection will quickly hide itself among the vegetation or in eroded crevices within the stream-bank wall. Because of the current, *Gerris remigis* is more active than other water-striders in terms of movement. The extent to which it drifts is limited because of its active movement.

Like other water-striders, *Gerris remigis* is predatory on land-dwelling insects caught in the water, as well as emerging insects (that have an aquatic larva life stage). Cannibalism is not uncommon, especially when food supply is low.

Individuals of this species are often seen in aggregations, especially in the fall prior to

overwintering. *Gerris remigis* overwinters in the adult stage, on land, and in leaf litter, under rocks, and debris (Stonedahl & Lattin 1982). Overwintering sites are, generally, near water.

The number of generations per year varies with climate. In Alberta, *Gerris remigis* is univoltine; eggs are laid in spring, nymphs mature in the summer, and adults overwinter in the fall. Adults are usually encountered on the water surface from late-April to mid-June and from mid-August to late-September. Again, this depends on climatic conditions. In milder climates, *Gerris remigis* can be bivoltine or trivoltine (Torre-Bueno 1917), and possibly polyvoltine in tropical regions (Stonedahl & Lattin 1982). The eggs of *Gerris remigis* are oblong, approximately 1 · 6 millimetres by 0 · 5 millimetres (Stonedahl & Lattin 1982). They are laid, in groups, on floating debris, such as sticks, leaves, and grass. Maturation time varies with climate, but is about a fortnight. There are five larval instars, and development time is, again, highly variable with climate.

Gerris remigis, overall, is an excellent research animal. It is a robust insect that is agile and graceful on the water. Because of its general availability, large size, and uncapricious behaviour, it is a reliable laboratory or field animal to study.

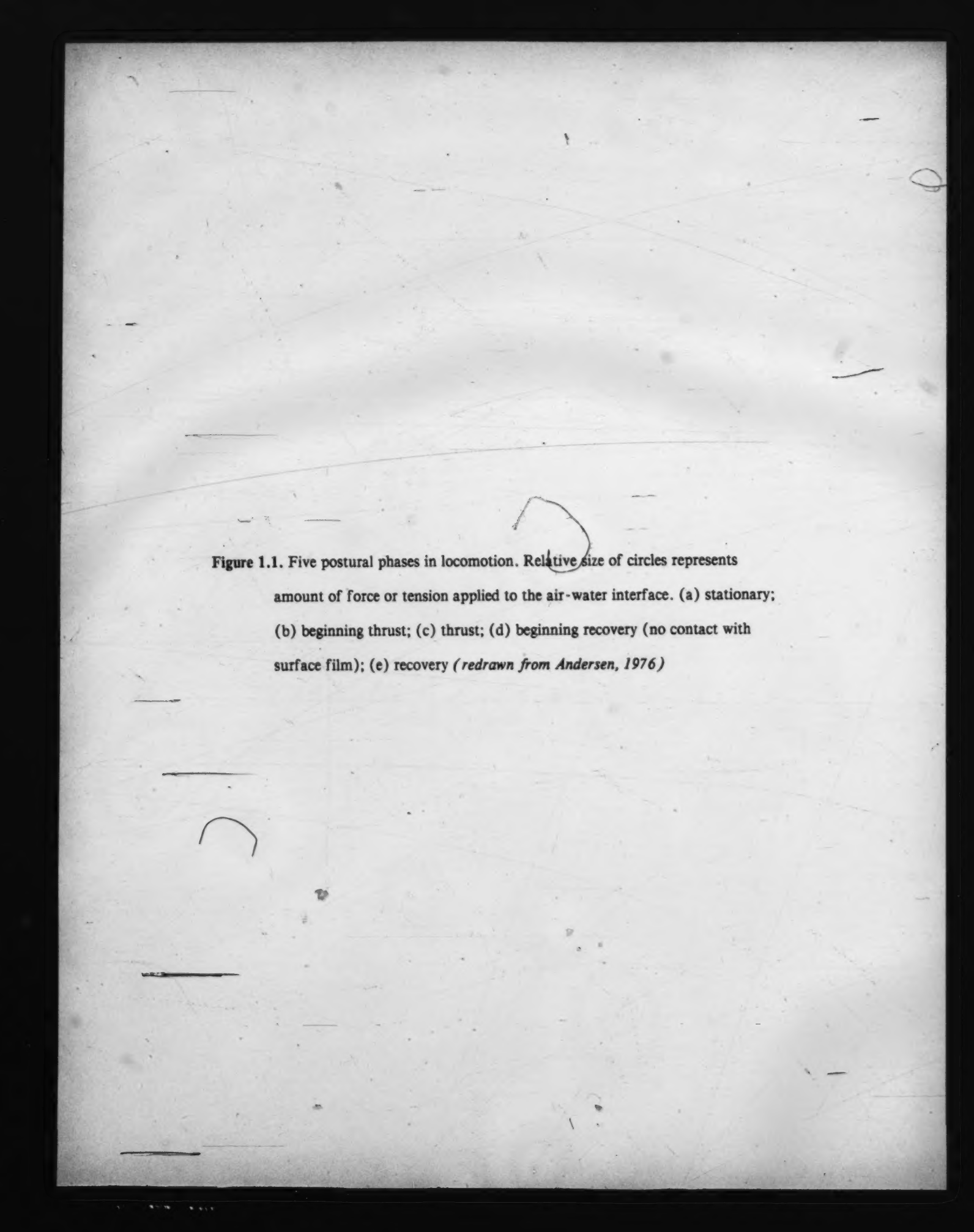
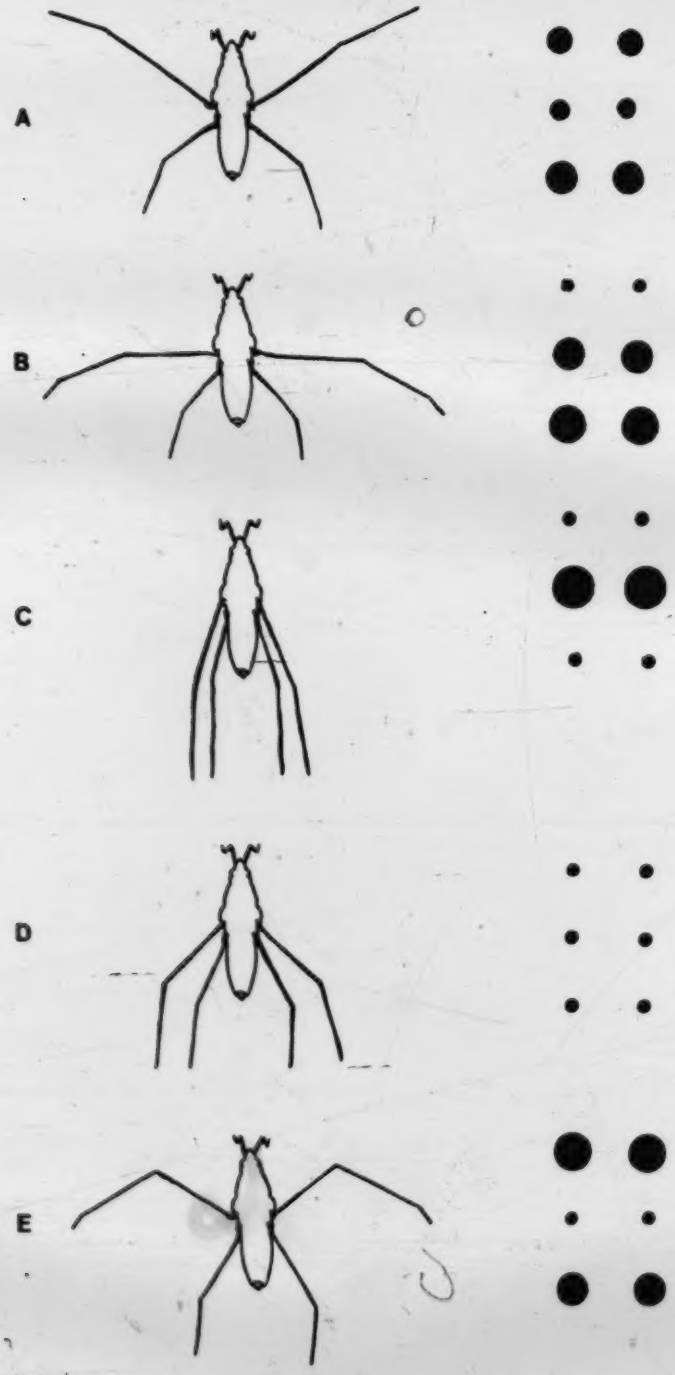


Figure 1.1. Five postural phases in locomotion. Relative size of circles represents amount of force or tension applied to the air-water interface. (a) stationary; (b) beginning thrust; (c) thrust; (d) beginning recovery (no contact with surface film); (e) recovery *(redrawn from Andersen, 1976)*



1-1

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2. A Catalogue of Cuticular Structures on the Legs of *Gerris remigis*

2.1 Introduction

Semi-aquatic Hemiptera, to which *Gerris remigis* belongs, have become most successful in adapting to life on the water surface. They are adapted to avoid wetting of the body, by positioning their body away from the water; and by possessing a hydrofuge macro-hair layer (Andersen 1976, 1977). At the same time, in order to remain on the water surface and for locomotive purposes, semi-aquatic Hemiptera require greater contact with the substrate (i.e., an increased surface area) than is necessary for terrestrial insects with their substrate. Thus, changes in leg structure and orientation have evolved mainly through the caudad positioning of the acetabulum and coxa (Andersen 1982; Bowdan 1976; Popov 1971). For detailed information on life history and adaptations of semi-aquatic Hemiptera concerning life on the water surface, the reader is referred to Andersen (1982) and Popov (1971). Much work has been done on locomotion and functional anatomy of legs, including cinemaphotographic analyses and biomechanical analyses (Andersen 1976; Bowdan 1976, 1978a, 1978b; Brinkhurst 1960; Darnhofer-Demar 1968, 1969, 1977; Murphey 1971a; Rensing 1962).

Another adaptation of some semi-aquatic Hemiptera is the ability to perceive water surface waves as signals, enabling them to detect potential predators, prey, and conspecifics. Most studies concerning wave perception have been done on three genera: *Notonecta* — an aquatic hemipteran (Markl and Wiese 1969; Wiese 1972; Wiese and Schmidt 1974); and two semi-aquatic Hemiptera *Velia* (Meyer 1971a, 1971b; Rensing 1962) and *Gerris* (Lawry 1973; Liche 1936; Murphey 1971a, 1971b; Rensing 1961; Walker 1983; Wilcox 1972, 1979, 1980; Wilcox & Kashincky 1980). Studies involved behavioural experiments, ablation experiments, histology, and electrophysiology.

According to Rensing (1961), *Gerris lacustrus* responds to ripples in amplitude of less than 4 μm and greater over frequency ranges of 20 – 500 Hz (peaking at 150 – 200 Hz). Wilcox (1979) found *Gerris remigis* responsive to ripples of 1 μm and greater over frequency ranges of 20 – 50 Hz (responding to prey) and 85 – 90 Hz (high frequency sex recognition signals; he found that only males signal and only males respond to HF signals). Females of *Rhagadotarsus kraepelini* also respond to signals (Wilcox 1972, 1980) as may other gerrid species.

At present, the location, structure, and function of the sense organ involved in surface wave location is unknown. Lawry (1973) noted long trichobothria located on the femur, trochanter [*sic*], and tarsus of *Gerris remigis*. He hypothesised that these hairs, especially those located on the trochanter and tarsus, sense waves on the water surface. Lawry also demonstrated that ablation of trichobothria or lacquering trichobothria led to a reduced response to prey. This hypothesis is a bit ambiguous because (*i*) the illustration in the paper is mislabelled, the tibia being labelled the trochanter, and the trochanter not being labelled at all; the text appears to follow the same pattern; and trichobothria are, indeed, found on the trochanter; (*ii*) he did not explain his ablation and lacquer experiments in sufficient detail, mentioning that results of behavioural experiments involving untethered lacquered animals are reported elsewhere. I have been unable to find this paper.

Murphey (1971a, 1971b) published a thorough study of orientation to prey, involving motor control and sensory aspects. This included a set of ablation experiments, the results of which suggest that a sense organ in the tibial-tarsal joint (e.g., a stretch receptor) is omni-directional, and that the system possibly functions by determining the receptor leg nearest the source of ripples. Murphey did not, however, confirm the location and structure of the presumtive sense organ through histological techniques or electrophysiology.

Murphey's conclusions seem reasonable since detection of surface vibrations by a Type II mechanosensitive organ is known to occur in *Notonecta glauca* (Wiese 1972) and — in part — the Sand Scorpion *Paruroctonus mesaensis* (Brownwell 1985). However, trichobothria cannot

be excluded since some invertebrates use these as detectors of air vibrations (Schuh 1975). A combination of these two sense organs and or another as yet unknown sense organ could be responsible for surface wave detection.

In this study I examine the external cuticular structures found on legs of *Gerris remigis*. Possible functions of sense organs and other structures are discussed (especially those that may detect water surface waves). Differences between males, females, and nymphs are noted as well as differences between my results and those of previous workers.

2.2 Materials and Methods

Specimens of *Gerris remigis* Say were collected, in late August and early September 1984, from the George Lake Field Station (Department of Entomology, The University of Alberta) located 16 kilometres west of Busby, Alberta.(Voucher specimens are deposited in the University of Alberta Strickland Museum.) Specimens were overwintered in plastic containers filled with *Sphagnum* sp. at 4° C, and were brought out as needed.

Preparation for scanning electron microscopy involved removing legs from dead specimens with scissors and soaking them overnight in detergent and hot water. They were then put into fresh detergent solution, sonicated for 45 seconds and rinsed in hot water several times for at least fifteen minutes each. A dehydration series followed, consisting of: 30% ethanol (30 minutes), 50% ethanol (one hour), 70% ethanol (two hours), and 95% ethanol (overnight). Legs were air-dried, and mounted on stubs using silver conducting paint, and gold coated using a Nanotek Samprep 2 Sputter Coater. Observations were made using a Cambridge Stereoscan 250 scanning electron microscope.

Preparation of specimens for light microscopy involved taking specimens preserved in alcohol, and macerating them in a KOH solution for several hours and then mounting legs in glycerin.

Hair densities were estimated by taking several counts from various electron micrographs of

Gerris remigis legs. Mann-Whitney U test was used in statistics.

The study is based on seven males, seven females, and seven nymphs (of different instars).

2.3 Results

As in other gerrids, the three leg-pairs of *Gerris remigis* differ markedly in structure and function (see figure 2.1). The prothoracic legs are shortest and used for support, grasping of prey, and in males, for grasping the female during copualtion. The prothoracic coxa is ventrally positioned close to the midline. The trochanter is more distinct and separate than in other leg-pairs. The femur is thickened, narrowing slightly at the distal end. The tibia is slightly shorter than the femur, and the tarsus is composed of two articles, the distal one being slightly longer. The tarsal claws are prominent and hook-shaped, arising from a terminal cleft. Arolia are reduced, the ventral arolium being broad-based.

The mesothoracic legs are longest of the three leg-pairs (see figure 2.1). They serve as the main propulsive organ for locomotion; the increased length — due to prolongation of segments — enhances the impetus of the stroke (Andersen 1982). The coxa is located posteriorly (close to the metathoracic coxa), and projects caudad from its acetabulum (which is situated laterally on thorax) (see figure 2.2). The coxa is only slightly elongated on its latero-ventral edge, not as elongate as Andersen (1982) suggests for most members of the family. The trochanter is larger than those of the other leg-pairs; this is due, in part, to the attachment of extrinsic leg muscles M46 and M47 (Andersen) or M52 (Guthrie) to tendons on the trochanter (see figure 2.3) (Andersen 1976; Guthrie 1961). The mesothoracic femur is longer and slightly thicker than that of the metathoracic femur. The distal end of the mesothoracic femur has two small lobes that curve caudad (see figure 2.1). The tibia is elongate, but shorter than the femur; it tapers distally, the distal end, however, is slightly enlarged. The tarsus is two-segmented, the first tarsomere being much longer than the second. The pretarsus is reduced; claws are curved but

smaller than prothoracic claws.

The metathoracic legs — as with the mesothoracic legs — are elongate and slender (see figure 2.1). They too are used in propulsion, but also give support, especially during the retraction phase of a stroke by the mesothoracic leg. The coxa projects caudad from the acetabulum, which is situated more dorsally on the pleuron than the mesothoracic acetabulum (and coxa) (see figure 2.2). The metathoracic coxa and trochanter are smaller than those of the mesothoracic leg; the trochanter, as well, is not as curved. The femur and tibia are not as long as the mesothoracic femur and tibia. The tibia is longer than the femur. The shape of the femur and tibia is similar to that of the mesothoracic leg. The tarsus is two-segmented, the first tarsomere, again, being longer. The metathoracic tarsus is shorter than the mesothoracic tarsus. The metathoracic pretarsus is reduced; and claws are curved.

The cuticular surface is finely folded or tuberculated, but there are areas where it is smooth (see figures 2.4 & 2.5). Moreover, there is a rather elaborate microsculpture around various leg joints that differs greatly from the rest of the leg cuticle (see figures 2.6 & 2.7).

Located on the cuticular surface on all segments of the three leg-pairs are, what appear to be, small pores. They are distributed unevenly with no apparent pattern other than being concentrated in groups (see figures 2.8 & 2.9). In addition, on the proximal part of the leg (coxa, trochanter, and femur) many of the pores are in depressions (which will be referred to as pit pores) (see figure 2.10). From the distal portion of the femur to the proximal portion of the tibia there is a gradation from pit pores to just pores.

There are two major hair layers in *Gerris remigis*; (*i*) a micro-hair layer composed of unsocketed cuticular outgrowths and (*ii*) a macro-hair layer of socketed, flexible hairs (Andersen 1977). While the body is densely covered with both types of hair layers, the legs have a very reduced micro-hair layer; in fact, it is exceptional to find micro-hairs on the legs (see figures 2.4 & 2.5).

The macro-hair layer density on the leg is approximately 250 hairs per square millimetre. Hair length ranges from 25 μm to 40 μm, the base width is approximately 2 μm (with the exception of setae whose bases average 12 μm) (see figures 2.4 & 2.5). The hairs are tapered to a point and are striated (see figure 2.8); they are inserted at a 50° to 60° angle relative to the cuticle. A slight modification of hairs on the tarsi and the ventral side of the mesothoracic and metathoracic tibiae is found where they are inclined at 35° to 50° and their apices are curved to form an 'L-shape' (see figures 2.12 & 2.13). Another feature of the macro-hair layer occurs on the ventral side of the prothoracic tarsus where hairs are much shorter and less dense (see figure 2.16).

Specialised macro-hairs — setae — are found on the femur, tibia, and tarsus of metathoracic and mesothoracic legs. They are about the same length as other macro-hairs, striated, and socketed; they differ mainly in their width (see figures 2.14 & 2.15).

A grooming comb is located on the distal end of the prothoracic tibia (see figures 2.16 & 2.17). It consists of about 30 hairs, 40 μm in length, densely packed into a row. At the base of each hair there appears to be a small pore (see figure 2.18). The comb is almost certainly used for grooming of hair layers, proboscis, etc., but the pores hint of a sensory function.

There are various mechanosensitive organs on legs of *Gerris remigis*, some of which are found on all three leg-pairs. The most widely distributed one is a small, 2 – 4 μm, campaniform sensillum, sometimes round and sometimes oval with a 'tail', which is found on all three leg-pairs and presumed to be on all segments (see figures 2.19 & 2.20) (This sensillum has not been found on the coxa.)

A campaniform sensillum similar to the one described above differs in being tear-drop shaped, 8 μm in length, and set into a cuticular swelling (see figure 2.21). This sensillum is found in a linear arrangement of three on the ventral side of the tibia near the femoral-tibial joint of the prothoracic leg (see figures 2.22 & 2.23). On the metathoracic leg in the same area one

companiform sensillum was noticed on one specimen.

The third type of campaniform sensillum is quite distinctive in shape and very specific in location. It is spindle-shaped, 30 μm in length and 3 μm wide (see figure 2.24). It is located on the anterior and posterior sides of the baso-trochanter on all three leg pairs in groups of four, three, three, and three respectively (see figure 2.25).

A sensory hair plate of six trichoid hairs 50 μm long is located ventrally on the baso-trochanter of the mesothoracic leg (see figures 2.26 & 2.27). A similar hair plate may exist on the metathoracic leg, but this has not been confirmed.

On the trochanter and femur of all three leg-pairs are trichobothria. There are three on the trochanter and seven, perhaps more, evenly spaced down the length of the femur on the ventral and lateral sides (see figure 2.28). They are 2 μm wide at the base and 120 – 150 μm in length. The base of each trichobothrium is inserted in a bothrium with no trichoma, and at a right angle to the cuticle (see figure 2.29). At the distal end of the tarsus are three tarsal hairs similar in structure to the trichobothria described above (see figure 2.27). Lawry (1973) refers to these as trichobothria, which they may be.

Mesothoracic and metathoracic trochanters and femora of the male are covered with basiconic type sensilla (see figure 2.31 & 2.32). They are distributed on the ventral side of the trochanter and femur. The base width is approximately 20 μm and the length is approximately 25 – 35 μm; it is inserted on a dome-like base (see figure 2.33). The surface of the sensillum is slightly grooved and appears to contain small pores (see figure 2.34).

There is a significant difference in the number of basiconic sensilla on the mesothoracic trochanter and femur as compared to the metathoracic trochanter and femur ($U=0$; $n_1=5$; $n_2=10$; $p<0 \cdot 002$). The mesothoracic trochanter has $x=33 \cdot 80 \pm 2 \cdot 80$ sensilla and the femur has $x=80 \cdot 80 \pm 5 \cdot 80$ sensilla, for a total of $x=114 \cdot 60 \pm 3 \cdot 70$ sensilla. The metathoracic trochanter has $x=9 \cdot 40 \pm 3 \cdot 20$ sensilla and the femur has $x=22 \cdot 60 \pm 4 \cdot 80$ for a total of $x=$

32 sensilla ± 5 · 01.

2.4 Discussion

For a review of leg structure and function of the Gerridae, the reader is referred to Andersen (1976, 1982); Bowdan (1976, 1978a, 1978b); Brinkhurst (1960); Darnhofer-Demar (1968); Matsuda (1960); Rensing (1962).

The presence of small groups of pores on the cuticular surface of the legs of *Gerris remigis* has not been mentioned in the literature. I hypothesise that they secrete some type of waxy hydrocarbon which is spread onto the cuticle and hairs to enhance the hydrofuge property of the legs. It is not known why the more proximal groups of pores are contained in depressions; perhaps an increased surface area would help in dispersing the substance over a larger area (the proximal part of the leg being larger). An observation that helps support this hypothesis is that of subcuticular 'glandular-like' tissue with ducts running to the surface (see figures 2.35 & 2.36). This structure was noticed when doing routine SEM work and casually looking at a cut edge of a leg.

In order to test this hypothesis it must be ascertained whether these surface pits are actually pores, whether the subcuticular material is glandular, and whether the putative pores and glandular tissue are in any way connected. Sectioning of the leg and histological staining of sections for glandular material will help elucidate these structures and their functions.

The macro-hair layer is strongly hydrofuge so there is a high deformation angle of the surface film. Surface tension forces repel hairs (and legs) away from the water surface (Andersen 1976). The claws, however, are able to pierce through the water surface allowing traction in movement (Andersen 1976). These are two major properties that enable *Gerris remigis* to live on the water surface.

The hair layer is designed to optimize hydrofuge properties and to protect the insect from water. L-shaped hairs on the ventral side of the mesothoracic and metathoracic tibiae form a barrier by overlapping each other distally; the inclination of the proximal part of the hairs acts as a suspension; this system allows greater pressure to be applied without wetting of the insect (Andersen 1976; Thorpe and Crisp 1947).

Moreover, the density of the hair layer is important in protecting the insect from wetting. Hair density is varied on each of the three leg-pairs, with an average density in *Gerris remigis* of 250 hairs per square millimetre. This estimate of hair density differs by an order of magnitude from that of Andersen (1976) who estimated density of the most common [macro] hair type (20 – 40 μm long) on legs of *Gerris* ranges from 4,000 – 16,000 per square millimetre. Comparisons of electron photomicrographs in Andersen's work and electron photomicrographs from this study show little difference in hair density — i.e., approximately 250 hairs per square millimetre. I, therefore, believe Andersen's estimations are inaccurate.

The hydrofuge property of the macro-hair layer, while effective, is not permanent; wetting can occur easily when hairs are covered with debris. Grooming is essential for maintenance of the hydrofuge property by keeping hairs in proper alignment, and removing water, oils, and debris from hairs. The grooming comb plays a major rôle in grooming of prothoracic and mesothoracic legs, the proboscis, and parts of the head and body (Andersen 1976).

Close examination of the base of each hair that comprises the comb, reveals a small knob or pore (see figure 2.18). This suggests possibly two additional functions of the structure : (*i*) the pore secretes a substance (possibly in aiding of hydrofuge maintenance) or (*ii*) the pore is an ecdysial scar (commonly seen on campaniform sensilla) and the grooming structure functions as a mechanoreceptor detecting stress of cuticle or movement. Again, histological work needs to be done to determine whether or not the grooming comb has a secondary function.

The number and distribution of small campaniform sensilla (figure 2.17) found on the legs is

not surprising. Most insects have campaniform sensilla on their legs to detect movement of joints and stresses and strains incuticle. Andersen (1982) even describes a campaniform sensillum on the proximal end of the tarsus as part of the Gerromorpha ground plan, as well as a grouping on the trochanter, which will be discussed below.

The three elongate campaniform sensilla found on the ventral side of the prothoracic tibia (see figure 2.22) may be used in prey-capture or feeding (e.g., sensing prey position). While it is noted that one campaniform sensillum was found on the ventral side of the metathoracic tibia in one preparation, they otherwise have not been found on mesothoracic and metathoracic tibiae (legs not directly used in feeding).

Andersen (1982) states that, in Gerromorpha, part of the ground plan consists of spindle-shaped campaniform sensilla in groups on the trochanter (see figure 2.24). The most plesiomorphic number is most likely eleven; five dorsally located and six ventrally located (Andersen 1982). However, in Gerridae there are thirteen (seven dorsally located and six ventrally located) (Andersen 1982).

In *Gerris remigis* there are thirteen campaniform sensilla; the two medial groups are more difficult to see using scanning electron microscopy. (Macerated specimens and light microscopy were used to identify these seven campaniform sensilla.) These thirteen campaniform sensilla possibly supply information concerning leg position with respect to the body.

The type of hair plate found on the mesothoracic trochanter has been briefly described in other gerrids (Andersen 1982). The hair plate is difficult to locate. It was found only on the mesothoracic trochanter; a more thorough search must be carried out to determine if it is present on the prothoracic and metathoracic legs. There are several possible functions of the sensory hair plate, the main one, being detection of leg position (as is the trochanteral hair plate of the cockroach (Wong and Pearson 1976)).

Trichobothria are found in many Hemiptera; they are believed to play a rôle in detecting

auditory, tactile, and vibratory cues (Schuh 1975). In *Gerris remigis* there is no trichoma surrounding the dome-like bothrium. Lack of this trichoma and a right angle orientation to the cuticle suggest that the sensillum is omni-directionally sensitive. (Trichobothria are the sensory hairs to which Lawry (1973) refers as receptors of water vibration.)

All of the above mentioned Type I mechanosensitive sense organs could function as water vibration receptors. Electrophysiological studies in conjunction with more behavioural studies need to be done in order to identify the actual receptor. Detection of water surface signals probably involves both Type I and Type II mechanosensitive organs — e.g., those proposed by Lawry (1973) and Murphey (1971a) respectively. These sense organs may be located near the tibial-tarsal joint (Murphey 1971a).

The basiconic sensilla found on male mesothoracic and metathoracic trochanters and femora have not been described previously. At the light microscopic level they are virtually indistinguishable from setae. And, most major studies of gerrid legs have used species other than *Gerris remigis*. A survey of twenty-five species in seven genera showed only three other species that have basiconic sensilla on the legs (see chapter 3).

These sensilla are labelled 'basiconic sensilla' — inferring a chemosensory function — because they have the classic shape of a chemosensitive basiconic sensillum, and there are what appear to be small pores on the surface. (The pores are, however, very small and rather obscure (see figure 2.34).)

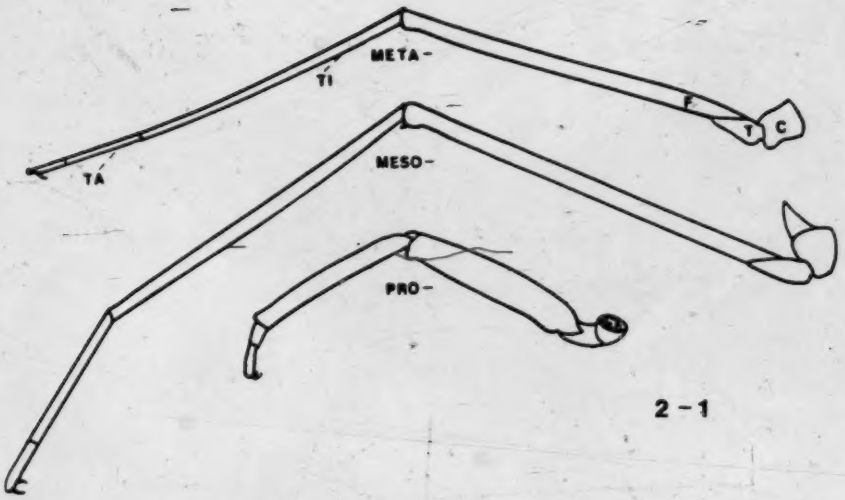
Since the sensilla are found only in males and only in adults, the most parsimonious hypothesis regarding their function would involve some aspect of mating behaviour (e.g., contact pheromone). At this point in the study, it is hypothesised that these are chemosensitive and detect some chemical cue emmitted by the female.

Figures 2.1 to 2.3. Illustrations. A, acetabulum; C, coxa; F, femur; M, muscle; T, trochanter; TA, tarsus; TI, tibia.

Figure 2.1. Three Leg-pairs. Prothoracic (PRO) Mesothoracic (MESO), and Metathoracic (META) legs.

Figure 2.2. Acetabula and Coxa. Ventral aspect showing orientation.

Figure 2.3. Extrinsic leg muscles. With respect to the coxa and trochanter. (labelled according to Andersen)



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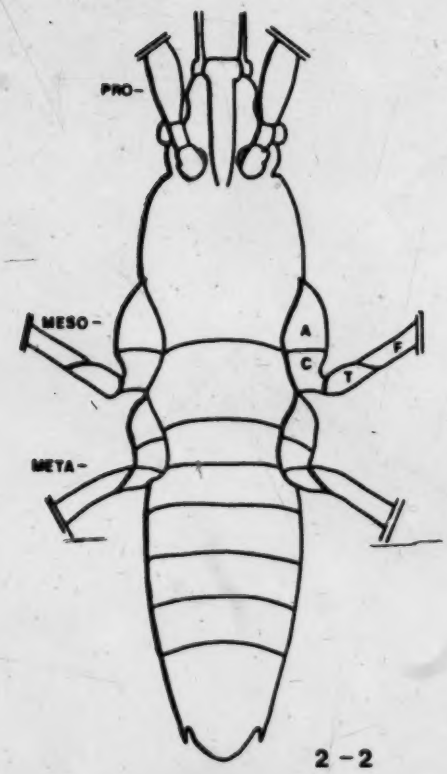

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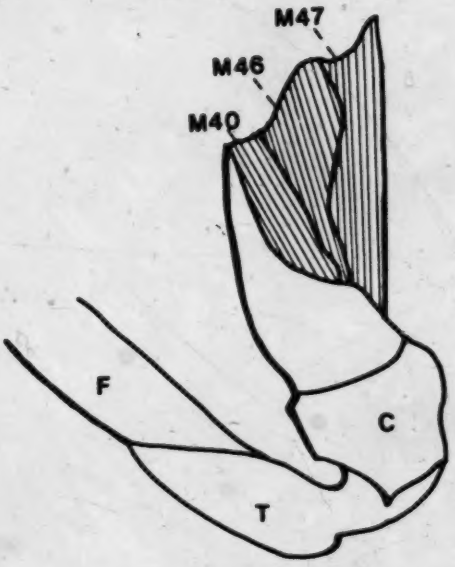

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Figures 2.4 to 2.9. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.4. Macro-hair layer on mesothoracic femur. Scale bar = 40μm.

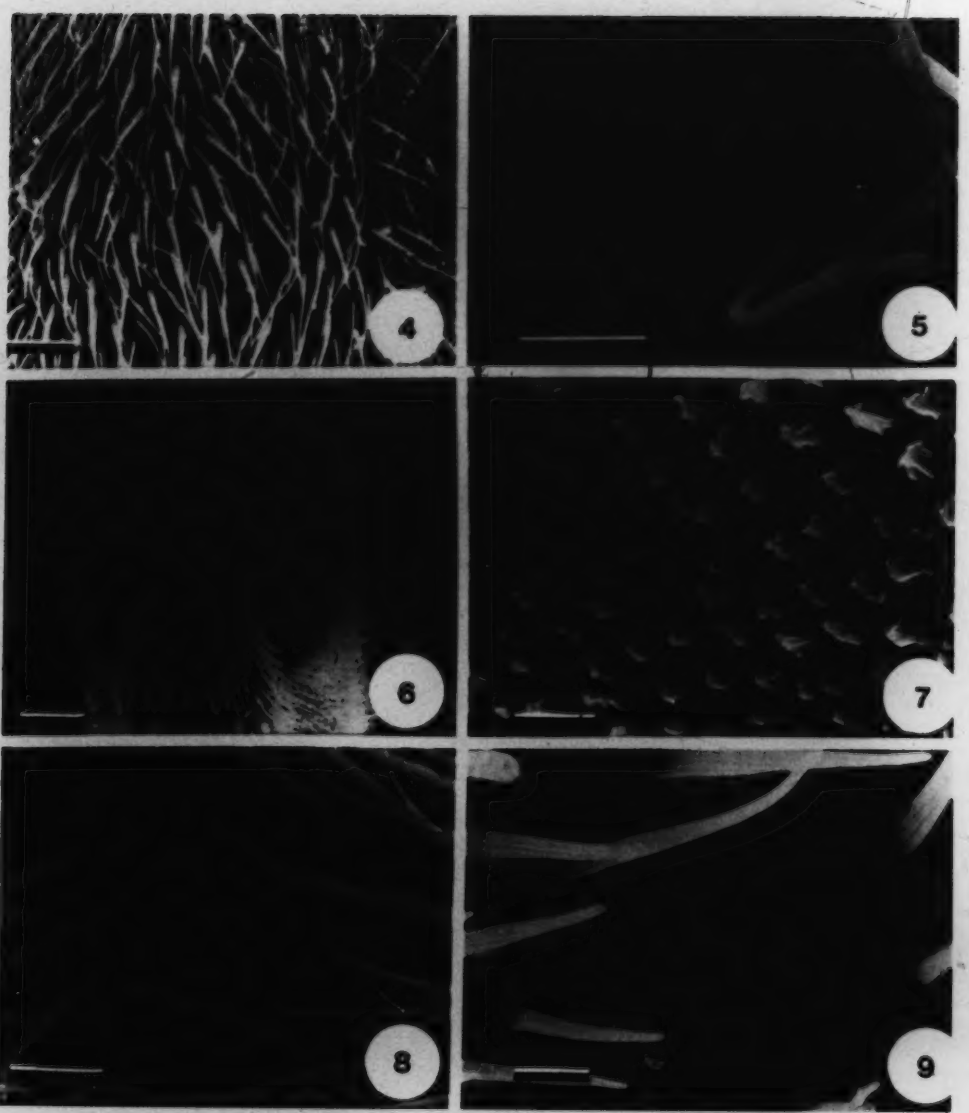
Figure 2.5. Macro-hair and micro-hair layers. Scale bar = 10μm.

Figure 2.6. Joint sculpture of femoral-tibial joint of mesothoracic leg. Scale bar = 100μm.

Figure 2.7. Close up of joint sculpture. Scale bar = 8μm.

Figure 2.8. Micrograph showing rough cuticle and pit pores. Scale bar = 10μm.

Figure 2.9. Close up of pores. Scale bar = 4μm.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Figures 2.10 to 2.15. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.10. Close up of pit pore. Scale bar = 4μm.

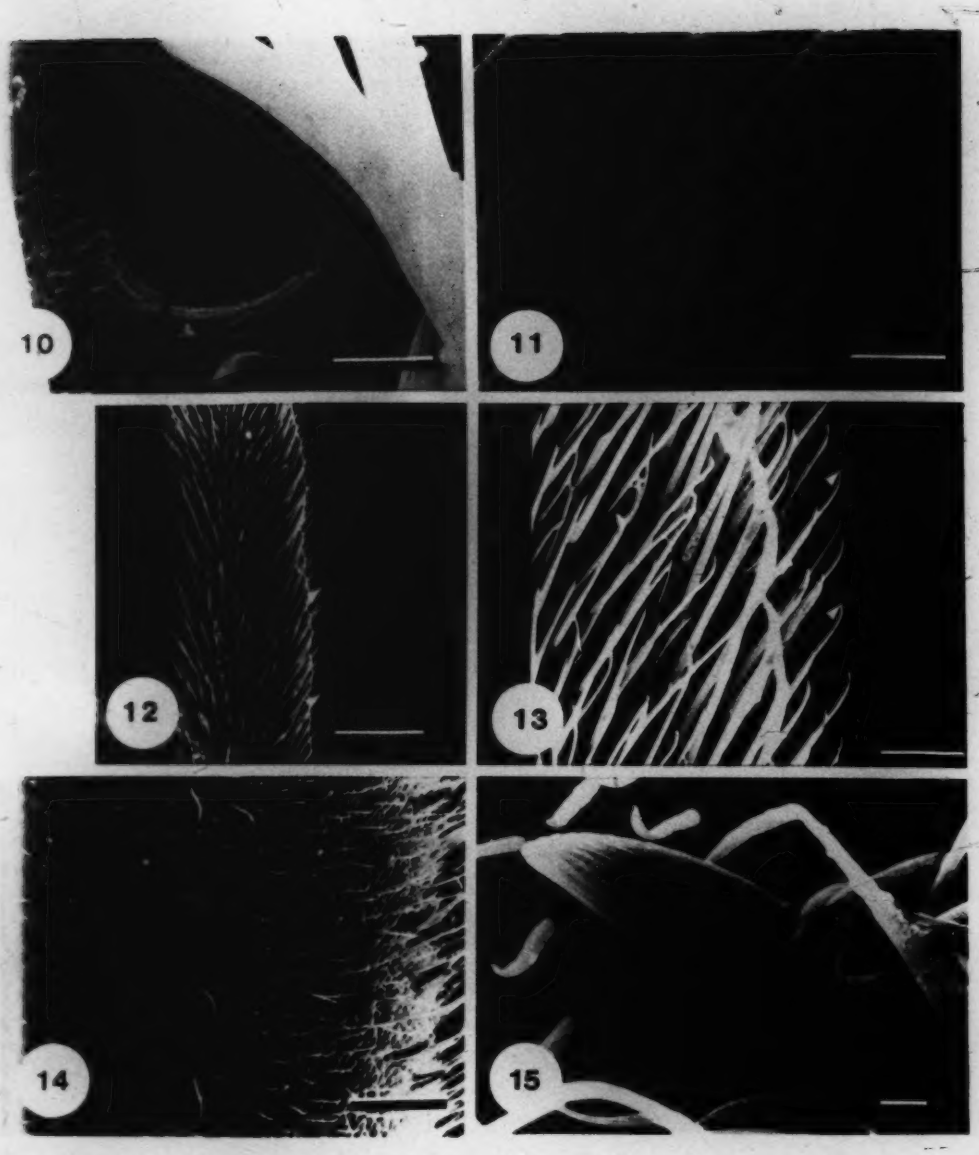
Figure 2.11. Micrograph showing striations on a macro-hair. Scale bar = 4μm.

Figure 2.12. Mesothoracic tarsus. Note L-shaped hairs. Scale bar = 80μm.

Figure 2.13. Close up of L-shaped hairs showing inclination. Scale bar = 20μm.

Figure 2.14. Macro-hair layer including setae on metathoracic femur. Scale bar = 80μm.

Figure 2.15. Close up of setae. Note base and striations. Scale bar = 4μm.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Figures 2.16 to 2.21. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.16. Prothoracic tibia and tarsus. Note grooming comb and sparse shortened macro-hair layer. Scale bar = 100μm.

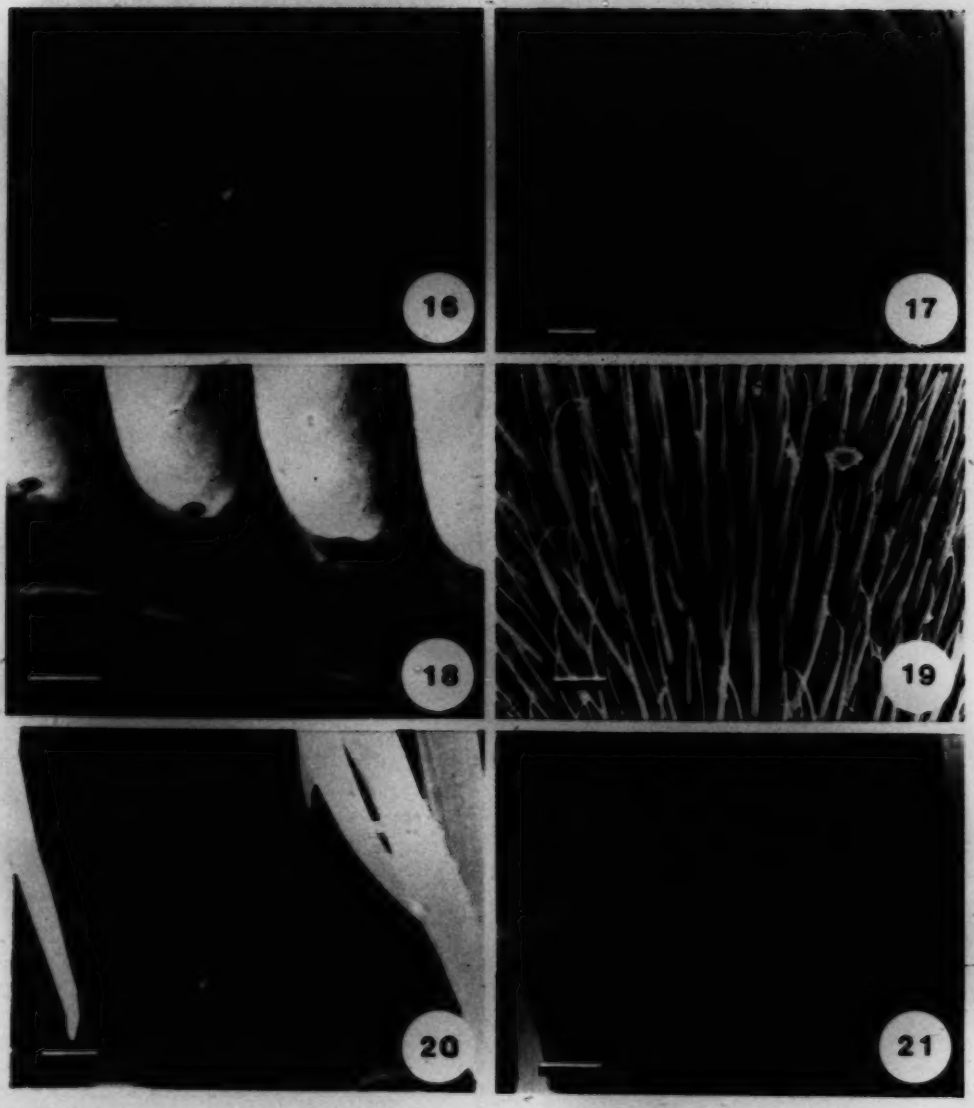
Figure 2.17. Close up of grooming comb. Scale bar = 20μm.

Figure 2.18. Knobs or pores at base of grooming comb. Scale bar = 2μm.

Figure 2.19. Location of small campaniform sensillum. Scale bar = 20μm.

Figure 2.20. Small campaniform sensillum with "tail". T, tail. Scale bar = 2μm.

Figure 2.21. Elongated campaniform sensillum set in cuticular swelling. On ventral side of prothoracic tarsus. Scale bar = 2μm.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Figures 2.22 to 2.27. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.22. Arrangement of three elongated campaniform sensilla. Scale bar = 20μm.

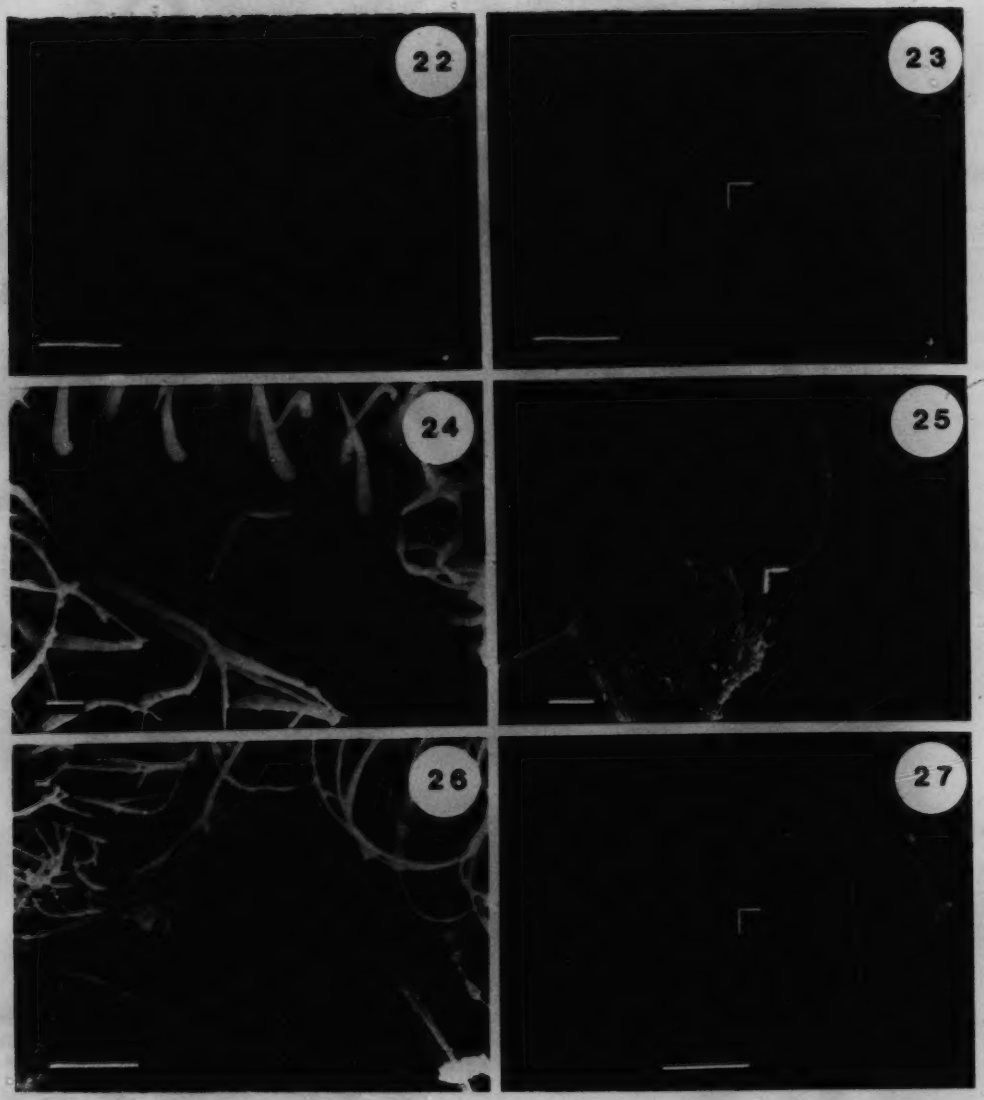
Figure 2.23. Location of three elongated campaniform sensilla. On prothoracic tarsus. Scale bar = 200μm.

Figure 2.24. Spindle-shaped campaniform sensilla. On mesothoracic trochanter. Scale bar = 4μm.

Figure 2.25. Location of the spindle-shaped campaniform sensilla. Scale bar = 200μm.

Figure 2.26. Sensory hair plate. Located on baso-trochanter of mesothoracic leg. Scale bar = 20μm.

Figure 2.27. Location of sensory hair plate. Scale bar = 400μm.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Figures 2.28 to 2.33. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.28. Trichobothria on femur and trochanter. Scale bar = 200μm.

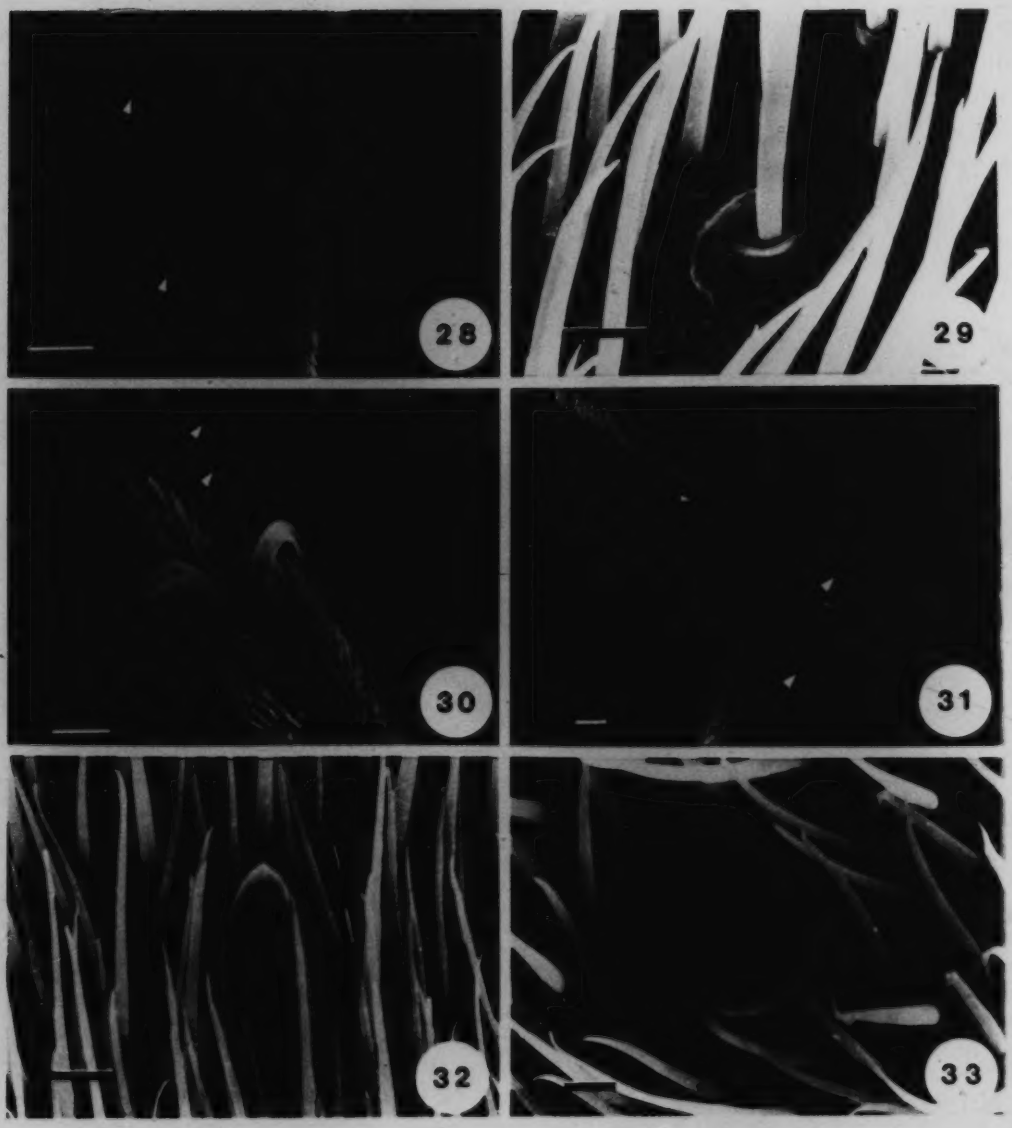
Figure 2.29. Trichobothrium base. Note dome-like bothrium. Scale bar = 8μm.

Figure 2.30. Metathoracic pretarsus showing three tarsal hairs. Scale bar = 50μm.

Figure 2.31. Basiconic sensilla on metathoracic femur and trochanter. Scale bar = 100μm.

Figure 2.32. Lateral view of basiconic sensillum. Scale bar = 10μm.

Figure 2.33. Dorsal aspect of basiconic sensillum. Note base on which sensillum is positioned. Scale bar = 8μm.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Figures 2.34 to 2.36. Scanning Electron Micrographs of *Gerris remigis*

Figure 2.34. Surface of basiconic sensillum. Note small pores. Scale bar = 400nm.

Figure 2.35. Subcuticular glandular material. Scale bar = 20μm.

Figure 2.36. Glandular material with ducts running to cuticular surface. Scale bar = 4μm.

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3. A Survey of Basiconic Pegs in Gerridae

3.1 Introduction

Gerridae have adapted well to life on the water-surface of streams and ponds. They have developed an extensive hydrofuge macro-hair layer as well as specialised body positioning with respect to the water to avoid wetting of the body (Andersen 1976,1977). In addition, to survive in this unique habitat, the Gerridae have evolved a sensory system that allows them to detect prey, to mate, and to set up territories and protect oviposition sites (Spence and Wilcox 1986, Vepsäläinen and Nummelin 1985, Wilcox 1979, Wilcox and Spence 1986). For the most part, the sensory system is sensitive to surface waves created by prey and conspecifics.

In the previous chapter I catalogued cuticular structures in *Gerris remigis*, a species that lives on streams, in order to gain insight into structures that possibly detect surface waves. On mesothoracic and metathoracic femora and trochanters of *Gerris remigis* males, I noted undescribed sensory structures shaped like basiconic sensilla. The shape suggests a chemosensory function, but, the function remains unknown. Here I describe the structure of these sensilla more fully, and present data on their distribution in the family Gerridae.

Lawry's (1973) scanning electron microscopy study of the legs of *Gerris remigis*, examined possible mechanosensitive hairs that detect surface waves. He does not mention basiconic sensilla on the trochanter or femur. It is possible that he based his studies primarily on females and thus did not find the sensilla; it is also possible that they were confused with setae that are located in the same area. Murphey (1971a, 1971b) worked on sense organs of *Gerris remigis* as well, but used behavioural experiments and electrophysiology. Other works that treat the Gerridae in general, such as Andersen's (1976, 1977, 1982), have little to say about *Gerris remigis*. When dealing with *Aquarius*, the subgenus to which *Gerris remigis* belongs, *Gerris*

najas the European sister species of *Gerris remigis* is usually used. Cuticular structures and morphology of *Gerris remigis* have, therefore, not been examined closely.

Since basiconic sensilla have not been previously described in the Gerridae, one is led to believe that these sensilla are present only in *Gerris remigis*, or that they exist in other species, but are, perhaps, confused with setae. The purpose of this study is to survey various species (males and females) in the family Gerridae for any basiconic sensilla similar in structure to those on *Gerris remigis* males. Species were chosen from a variety of lineages both closely related and distantly related to *Gerris remigis*.

This information may convey phylogenetic information which might relate certain aspects of mating behaviour, prey-capture, or feeding in the different groups.

3.2 Materials and Methods

Legs of twenty-five species in seven genera were examined: *Gerris argentatus* Schummel (Zurich, Switzerland); *Gerris buenoi* Kirkaldy (George Lake, Alberta); *Gerris comatus* Drake & Hottes(George Lake, Alberta); *Gerris conformis* (Uhler) (Black River, Ontario); *Gerris costae* (Herrich-Schaeffer) (Zurich, Switzerland); *Gerris gibbifer* Schummel (Zurich, Switzerland); *Gerris incognitus* Drake & Hottes (Moresby Island, British Columbia); *Gerris lacustris* (Linnaeus) (Zurich, Switzerland); *Gerris lateralis* Schummel (Zurich, Switzerland); *Gerris najas* (DeGeer) (Zurich, Switzerland); *Gerris odontogaster* (Zetterstedt) (Zurich, Switzerland); *Gerris pallidum* (Fabricus) (Zurich, Switzerland); *Gerris pingreensis* Drake & Hottes (George Lake, Alberta); *Gerris remigis* Say (George Lake, Alberta); *Gerris thoracicus* Schummel (Zurich, Switzerland); *Halobates micans* Eschscholtz (Gulf of Mexico); *Halobates proavus* B.-White (Pulau Tioman, Malaysia); *Limnoporus canaliculatus* (Say) (Chafley's Locks, Ontario); *Limnoporus dissortis* Drake & Harris (George Lake, Alberta); *Limnoporus notabilis* (Drake & Hottes) (Haney, British Columbia); *Limnoporus rufoscutellatus* (Latreille)

(Helsinki, Suomi); *Potamobates* sp. (Napo Province, Ecquador); *Rhagadotarsus kraepelini* Breddin (Australia); *Rheumatobates rileyi* Bergroth (Little Rock Lake, Ontario); *Trepobates subnitidus* Esaki (Little Rock Lake, Ontario). One male and one female were examined in all species except *Gerris remigis*, *Limnoporus notabilis*, and *Limnoporus dissortis* in which several animals were examined.(Voucher specimens of *Gerris remigis* are deposited in the University of Alberta Strickland Museum.)

Preparation for scanning electron microscopy involved removing legs from dead specimens with scissors and soaking them overnight in detergent and hot water. They were then put in fresh solution, sonicated for 45 seconds, and rinsed in hot water several times for at least fifteen minutes each. A dehydration series followed consisting of: 30% ethanol (30 minutes), 50% ethanol (one hour), 70% ethanol (two hours), and 95% ethanol (overnight). Legs were air-dried and mounted on stubs using silver conducting paint, and gold coated using a Nanotek Samprep 2 Sputter Coater. Observations were made using a Cambridge Stereoscan 250 scanning electron microscope.

In determining how closely or distantly related a species is to *Gerris remigis*, as well as other phylogenetic information, Andersen (1982) was used when dealing with the subfamilies of Gerridae, and Calabrese (1980) was used when dealing with the genera of the subfamily Gerrinae. However, Calabrese's classification was not followed completely; although unpublished, many workers in gerrid biology believe that *Gerris* and *Neogerris* are sister groups, and that *Limnoporus* is a sister group to them. I have used this 'unpublished' classification in this chapter. Concerning the genus *Limnoporus* I use the term '*Limnoporus rufoscutellatus* group' to group the three species *Limnoporus rufoscutellatus*, *Limnoporus dissortis*, and *Limnoporus notabilis*; I use the term '*Limnoporus canaliculatus* group' to group other members of this genus (*Limnoporus canaliculatus* was the only species of this group to be examined).

3.3 Results

Basiconic sensilla are distributed latero-ventrally on the mesothoracic and metathoracic femora and trochanters of *Gerris remigis* males. There are significantly more basiconic sensilla on the mesothoracic leg (approximately 115 as compared to 32 on the metathoracic leg). The sensillum has a base width of approximately 20μm and a length of approximately 25–35μm and is situated on a dome-like base (see figures 3.1–3.3). The sensillum is slightly striated with apparent small pores (which are similar in appearance to those found on chemosensitive hairs) (see figure 3.4). Basiconic sensilla were not found on fifth instar nymphs of either sex.

Sensilla similar in structure are found on the mesothoracic trochanter and femur of males and females of *Limnoporus dissortis*, *Limnoporus notabilis*, and *Limnoporus rufoscutellatus* (see figures 3.5–3.11). In *Limnoporus notabilis* base width of the sensillum is approximately 20μm and the length approximately 30μm; in *Limnoporus dissortis* base width is approximately 20μm and length approximately 40μm; in *Limnoporus rufoscutellatus* base width is approximately 12μm and length approximately 15μm. The sensilla are slightly grooved or striated and appear to contain small pores. Their bases, however, are not as pronounced as in *Gerris remigis*. They are distributed ventrally (not latero-ventrally, as is the case with *Gerris remigis*) along the trochanter and femur, extending further down the femur than in *Gerris remigis*.

3.4 Discussion

As mentioned above, specimens closely and distantly related to *Gerris remigis* were examined (see figures 3.12 & 3.13). Of all genera examined, only *Gerris* and *Limnoporus* have basiconic sensilla on their legs.

Of the fifteen species in the genus *Gerris* examined, eleven belong to the subgenus *Gerris (Gerris)* and four to the subgenus *Gerris (Aquarius)*. Of the four species, *Gerris remigis*, *Gerris conformis*, *Gerris najas*, and *Gerris pallidum* in the subgenus *Aquarius*, only *Gerris*

remigis appears to have the basiconic sensilla. All species in the subgenus *Gerris (Gerris)* lack them.

Basiconic sensilla were found on *Limnoporus dissortis*, *Limnoporus notabilis*, and *Limnoporus rufoscutellatus*; species belonging to the *Limnoporus rufoscutellatus* group. One other species, *Limnoporus canaliculatus*, belonging to the *Limnoporus canaliculatus* group was the only other species in the genus *Limnoporus* to be examined and was found not to have sensilla.

Limnoporus is a sister group to the *Gerris–Neogerris* group (see materials and methods). While they are closely related in comparison to the whole of Gerridae, the fact that basiconic sensilla are not found in *Gerris (Gerris)*, nor the *Limnoporus canaliculatus* group suggests that these sensilla were independently derived, once in *Gerris (Aquarius)* and once in the *Limnoporus rufoscutellatus* group (see fig 3.12). (This is supported by the finding of basiconic sensilla on male mesothoracic and metathoracic legs of *Gerris remigis*, and on both male and female mesothoracic legs in the *Limnoporus rufoscutellatus* group.)

Even in *Gerris (Aquarius)*, not all species have the basiconic sensilla. *Gerris najas*, the sister species of *Gerris remigis*, does not have basiconic sensilla on the legs of either sex. Since they are sister species it seems likely that *Gerris remigis* is the only member of the subgenus to have the basiconic sensilla, however, a more extensive survey needs to be undertaken to support this statement with any certainty.

The sensilla in both groups are probably homologous structures. The shape and distribution are roughly equal. Because *Gerris remigis* females do not have the sensilla, it does not mean that there are not specific genes — common to both groups — that code for the sensilla and are turned on in males of *Gerris remigis* and both sexes of the *Limnoporus rufoscutellatus* group.

The suggestion that they are independently derived raises the question of whether they are analogous in structure and function (physiologically as well as behaviourally). The appearance of basiconic sensilla in only one sex (as is the case with *Gerris remigis* males), and the lack of

these sensilla in fifth instar males and females, suggest a function in mating behaviour. The appearance of basiconic sensilla in both sexes of the *Limnoporus rufoscutellatus* group does not exclude the possibility of a function in mating behaviour.

Both groups have very similar mating behaviours in that they both signal, orientation of male to female is similar, as well as certain mating patterns (Vepsäläinen & Nummelin 1985; Wilcox & Spence 1986; Spence & Wilcox 1986). In *Limnoporus notabilis*, *Limnoporus dissortis*, and *Limnoporus rufoscutellatus*, mating behaviour is similar enough that the three species are able to inter-breed (Spence & Wilcox 1986; Wilcox & Spence 1986; Spence, personal communication). Males in these species can be territorial and produce spacing and courtship signals; females show no behavioural response to signals (Wilcox & Spence 1986b). Moreover, males are able to distinguish sex by presence or absence of spacing signals (Wilcox & Spence 1986b). Spence and Wilcox (1986a) have shown in *Limnoporus notabilis* and *Limnoporus dissortis* that pheromones are not necessary for sex determination.

The mating system in *Gerris remigis* differs in that males use a high frequency signal to identify other males (Wilcox 1979). (Again, only males signal and females show no behavioural response to the signals.) Moreover, *Gerris remigis* does not appear to be territorial nor does it signal from a signal site (Wilcox 1979). Wilcox (1979) has shown, by signal playbacks, that chemical cues (such as pheromones) appear to be unimportant.

Behavioural studies, thus far, show that a pheromone system is not necessary for sex recognition, however, they do not exclude a pheromone system as an aid in mating behaviour. Given the similarity in mating behaviour of *Gerris remigis* and the *Limnoporus rufoscutellatus* group, if a contact pheromone system were found in one species, it might be found in the other species. If the basiconic sensilla serve a function other than in mating behaviour, it is difficult to explain the apparent lack of basiconic sensilla in females of *Gerris remigis*. Given that basiconic sensilla are not found on females of *Gerris remigis*, and are not found on nymphs, it is most parsimonious to hypothesise that in *Gerris remigis* they function in mating behaviour

(regardless of whether they are chemosensitive or mechanosensitive structures and regardless of the function in the *Limnoporus rufoscutellatus* group).

Still, the structures in the two genera should be considered analogous in function — at least in part. The different groups may have behavioural mechanisms (using these receptors) that are specific to their group, but these differences are probably subtle variations of the basic physiological function of the receptors.

Given the similarities in mating behaviour of *Gerris remigis* and *Limnoporus rufoscutellatus*, the possibility of a mechanosensitive function for these sensilla in addition to a chemosensitive function must be considered. The basiconic sensillum shape and the subtle evidence of pores are a strong indication that the sensilla have a chemosensitive function. Also supporting this conclusion is the characteristic leg-rubbing movement of males on the thorax of females during mating (see chapter 5). However, I suggest that a mechanosensitive function cannot be ruled out for the following reasons: In *Gerris remigis* only the males possess the sensilla and only the males signal and respond to signals. In *Limnoporus rufoscutellatus* group both sexes have the sensilla and, reportedly, only males signal and respond to signals. (There is, however, some indication that females perceive the signals as well (Spence, personal communication).) Moreover, *Gerris najas*, the proposed sister species to *Gerris remigis*, does not have the sensilla, nor has a signal-mediated mating system been noted in studies of mating behaviour in this species. Another line of evidence supporting a mechanosensitive function is that behavioural experiments do not corroborate a pheromone-mediated mating system (see chapter 5).

If there is a connection between these sensilla and a mechanical signalling system, the presence of sensilla and signalling behaviour should be correlated throughout the family Gerridae. *Rhagadotarsus kraepelini*, a water-strider known to have an elaborate signalling system where both males and females signal (Wilcox 1979), does not, however, have basiconic sensilla on the legs. The lack of sensilla in *Rhagadotarsus kraepelini* does not support the signalling-mechanosensitive hypothesis. However, *Rhagadotarsus kraepelini* is quite distantly

related to *Gerris* and *Limnoporus*, belonging to the subfamily Rhagadotarsinae — it is possible that the signalling is controlled by different mechanisms in the two subfamilies. With current evidence, it seems unlikely that basiconic sensilla are connected with a mechanical signalling system in the Gerridae.

3.5 Conclusion

Basiconic sensilla have independently evolved at least twice in the Gerridae; both times in the Gerrinae. Once in *Gerris remigis* (subgenus *Aquarius*) and once in the *Limnoporus rufoscutellatus* group.

It is not known whether the structures are homologous and functionally analogous. They appear structurally analogous however. More studies involving the *Limnoporus rufoscutellatus* group are required in order to determine more completely the relationship of the sensory sensilla with regard to phylogenetic trends.

It is possibile that in the subgenus *Aquarius*, there are other species that have basiconic sensilla; and, that these sensilla are probably on the metathoracic and mesothoracic legs of males only. I also expected that *Gerris najas*, the sister species to *Gerris remigis*, would have the basiconic sensilla. However, they are lacking in this species.

Given the evidence presented above, I suggest, that in both the *Limnoporus rufoscutellatus* group and in *Gerris remigis*, these basiconic sensilla are homologous and have a functionally analogous rôle in mating behaviour most likely related to a pheromone system. Moreover, I believe that the presence of basiconic sensilla is not necessarily connected to the presence of signalling in mating behaviour. I base this hypothesis principally on the facts that only *Gerris remigis* males have the basiconic sensilla (not the females, and not fifth instar nymphs); and, that the sensilla structurally resemble very closely a "classic" chemosensitive sensillum.

Figures 3.1 to 3.4. Scanning Electron Micrographs of *Gerris remigis*.

Figure 3.1. Distribution of basiconic sensilla on mesothoracic trochanter of a male. Scale bar = 40μm.

Figure 3.2. Lateral aspect of sensillum. Scale bar = 10μm.

Figure 3.3. Dorsal aspect of sensillum showing base. Scale bar = 8μm.

Figure 3.4. Pores on the surface of a sensillum. Scale bar = 400nm.

Figures 3.5 to 3.6. Scanning Electron Micrographs of *Limnoporus notabilis*.

Figure 3.5. Distribution of basiconic sensilla on mesothoracic femur of a female. Scale bar = 100μm.

Figure 3.6. Lateral aspect of sensillum. Scale bar = 10μm.

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Figures 3.7 to 3.11. Scanning Electron Micrographs of the *Limnoporus rufoscutellatus* group.

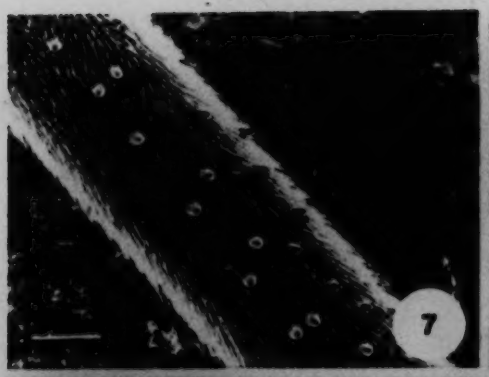
Figure 3.7. Distribution of basiconic sensilla on mesothoracic femur of *Limnoporus dissortis* female. Scale bar = 100μm.

Figure 3.8. Lateral aspect of sensillum on mesothoracic femur of *Limnoporus dissortis* female. Scale bar = 10μm.

Figure 3.9. Distribution of basiconic sensilla on mesothoracic femur of *Limnoporus rufoscutellatus* male. Scale bar = 40μm.

Figure 3.10. Lateral aspect of sensillum on mesothoracic femur of *Limnoporus rufoscutellatus* male. Scale bar = 4μm.

Figure 3.11. Pores on surface of sensillum of *Limnoporus dissortis* male. Scale bar = 400nm.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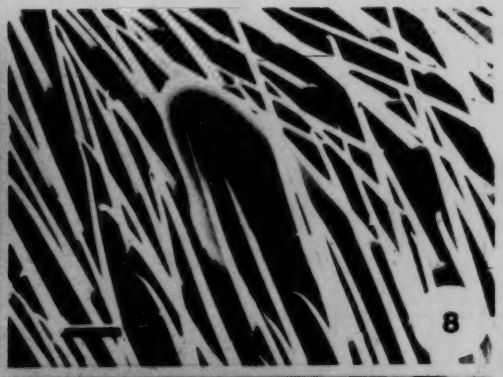
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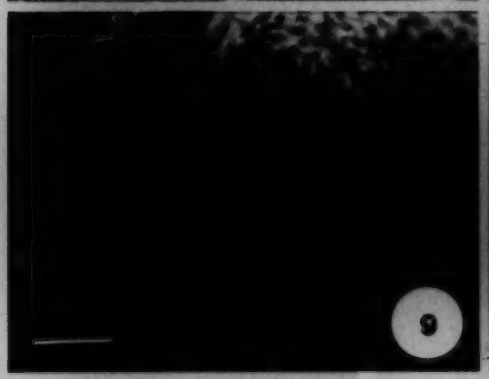
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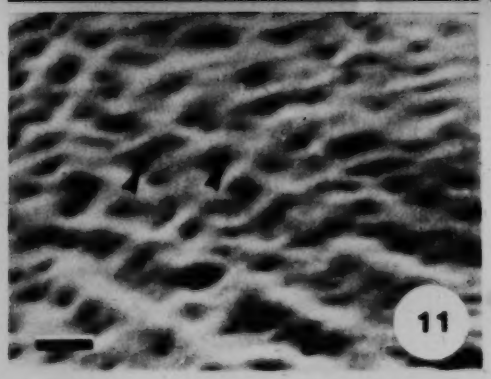
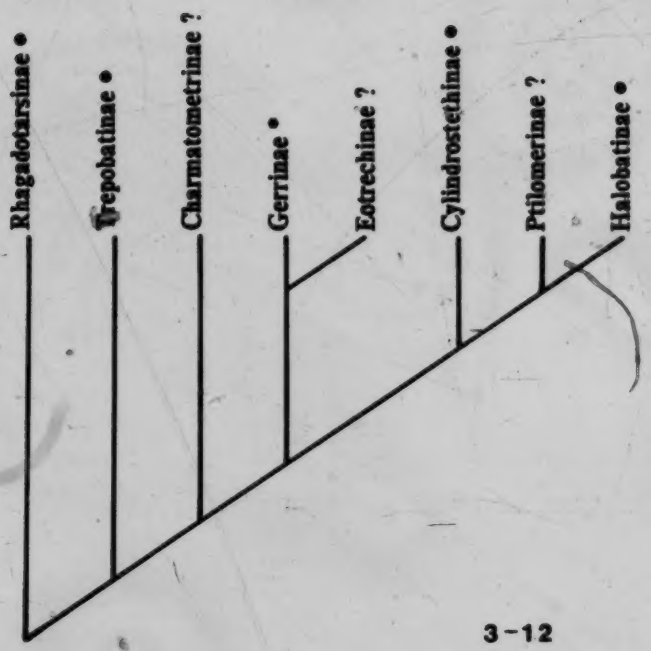

11

Figures 3.12 to 3.13. Cladograms of relationships within the Gerridae. (• denotes groups examined; * denotes groups where basiconic sensilla were found on some members; ? denotes groups not examined.)

Figure 3.12. Cladogram of relationships between subfamilies of Gerridae according to Andersen (1982).

Figure 3.13. Cladogram of relationships between genera in the subfamily Gerrinae according to Calabrese (1980).


Rhagadotarsinae •
Trepobatinae •
Charmatometrinae ?
Gerrinae •
Eotrechinae ?
Cylindrostethinae •
Ptilomerinae ?
Halobatinae •

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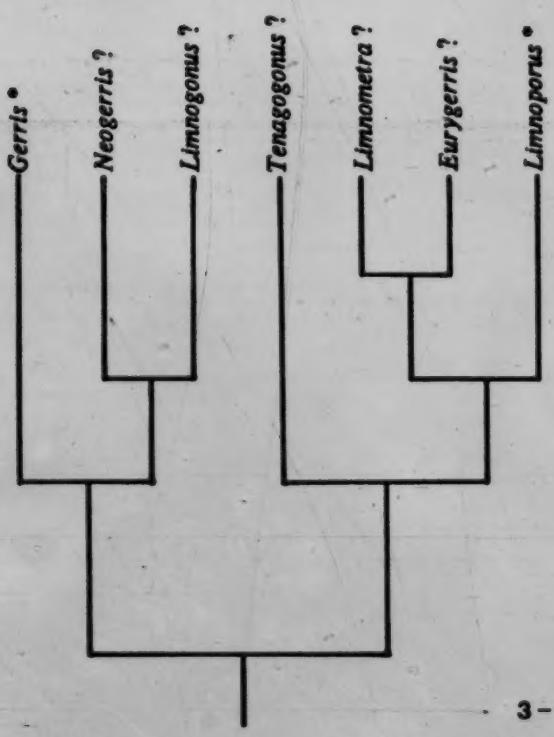
Gerris •
Neogerris ?
Limnogonus ?
Tenagogonus ?
Limnometra ?
Eurygerris ?
Limnoporus •

3-13

3.6 Literature Cited

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4. Histological Aspects of the Trochanter and Femur of *Gerris remigis*

4.1 Introduction

In Chapter 2 a basiconic sensillum is described. It is found on mesothoracic and metathoracic trochanters and femora of *Gerris remigis* males; approximately 115 on mesothoracic legs and 32 on metathoracic legs. A survey of other species revealed structurally similar basiconic sensillum sensilla in the *Limnoporus rufoscutellatus* group (see chapter 3), however, these sensilla are found in both sexes, and only on mesothoracic legs.

Scanning electron microscopy of the surface of the sensillum (of each species) reveals many pores. A basiconic sensillum shape and many pores on the surface suggest this sensillum is chemosensitive in function (Chapman, 1982; Zacharuk 1985). Moreover, there is some evidence that a contact pheromone-mediated mating system may exist in some species of Gerridae (species that have basiconic type sensilla on their legs)(see chapter 5).

The purpose of this study was two-fold (emphasis being placed on the latter): (i) To examine general tissue structure of the leg of *Gerris remigis*, especially emphasizing structures characteristic of Gerridae or Gerromorpha (e.g., cuticle, gland cells, and specific sensilla) to extend findings from SEM work. (ii) To locate and describe neurones and associated cells of the basiconic sensillum sensillum. If this type of sensillum is multi-innervated, it is even more likely that the sensillum is chemosensitive.

4.2 Materials and Methods

Males of *Gerris remigis* Say were collected from George Lake Field Station (Department of Entomology, The University of Alberta), located 16 kilometres west of Busby, Alberta; Little

Hornbeck Creek, located 20 kilometres west of Edson, Alberta; and Whitemud Creek in Edmonton, Alberta. (Voucher specimens are deposited in the University of Alberta Strickland Museum.) Some were overwintered in an incubator and legs fixed when needed. Others were brought directly from the field and legs fixed upon returning to the laboratory. Yet others were maintained in the laboratory and fed on vestigial-winged *Drosophila*, legs being fixed when needed. Both mesothoraic and metathoracic legs were examined. Approximately 40 legs of *Gerris remigis* were embedded in Epon and used in analysis.

Histology. Various methods of fixation, embedding, sectioning, and staining were employed. Most, however, were ineffective mainly because the cuticle is thick and the proteins heavily sclerotised. Embedding was done in paraffin, ester wax, paraffin-celloidin, and chitinase treated cuticle embedded in paraffin-celloidin. None of these methods provided adequate results; as a consequence obtaining serial-sections was difficult.

Staining of leg nerves with cobaltous chloride was also carried out. While effective fills were accomplished, the colour of the cuticle did not allow sufficient observation of leg nerves.

The method that provided best results was that of semithin sections of specimens embedded in Epon. Legs were removed from live insects, and, femora and trochanters cut into sizes that could be accomodated by Epon blocks. Specimens were pre-fixed in 5% gluteraldehyde in phosphate buffer (pH 7) for one hour at room temperature. After buffer rinses of 40 minutes, they were fixed in 2% osmium tetroxide and buffer for two hours at 4°C. Preparations were given several buffer rinses totalling 40 minutes; they were dehydrated in ascending concentrations of ethanol, starting at 70% (at 4°C). At room temperature, preparations were left in absolute ethanol for two changes of 20 minutes each. They were then placed in propylene oxide for two changes of 20 minutes each; and transferrred to a 1:1 mixture of Epon and propylene oxide, and, left overnight at room temperature. The following day specimens were placed in fresh Epon for two hours and then embedded in fresh Epon in moulds. Moulds were placed either in a vacuum oven for three hours or in a standard laboratory oven (set at 60°C)

for eight to ten hours. Blocks were cured for at least two days. Sections were cut on a Reichert OM U2 ultramicrotime using glass knives. Semithin sections (i.e., placed on light microscope slides) were cut at various thicknesses ranging from 900nm – 7μm. Most sections were cut in the range of 1 – 4 μm. Both, longitudinal- and cross- sections were cut.

Staining. Sections were stained with 0 · 5% Toluidine Blue O in a 1% Borax aqueous solution for about one minute (time varied with section thickness) on a hot plate at lowest setting. Slides were rinsed, differentiated in 98% ethanol for 15 seconds and permanent mounts were made using Canada Balsam or Euparal®.

Microscopy. Analysis of sections was made using a Leitz Wetzlar compound microscope, as well as, a Wild M20 compound microscope. Phase Contrast and Nomarski Optics were employed with many slides. Photographs were taken with a Wild M20 compound microscope and a Wild Photoautomat MPS 45.

Cell counts. Cell counts were obtained by identifying a basiconic sensillum with an apparantly large number of cells beneath it. Cells were counted within a 90μm linear space in the epidermal cell-layer. Then — within the same section — cells were counted within a similar 90μm linear space in the epidermal cell-layer where there was no visible evidence of mechanosensitive or chemosensitive structures save for socketed hairs (which are ubiquitous on the legs). Counts of basiconic sensilla and their surrounding cells, and the corresponding 'sensillum-free' area were carried out on 10 different sensilla. Non-parametric statistics were applied using the Mann-Whitney U test to test the significance of the counts.

4.3 Results and Discussion

4.3.1 General Histology

Figure 4.1 is of a cross-section at the trochanteral-femoral jont. Figure 4.2 is of a corresponding longitudinal-section. The thickness and degree of sclerotisation of the cuticle is obvious. The leg is divided into two compartments, the smaller one being the dorsal compartment. There are three major tracheal trunks (only one of which is contained within the longitudinal-section). There are three major nerve trunks visible adjacent to the tracheal trunks (again, only one is visible in the longitudinal-section), at higher magnifications individual nerve fibres can be seen. Cross-sections and longitudinal-sections of various muscles can be seen in both cross-sections and longitudinal-sections of the leg. Sarcosomes can be seen within the muscle fibres. In the interstitial spaces, trachea can be seen, as well as fat cells, connective tissue; and haemolymph. It should also be noted that the epidermal cell-layer is reduced and not easily distinguishable.

4.3.2 Epidermal cell-layer

Figure 4.3 shows the cuticle and epidermal cell-layer in a longitudinal-section. Complete cells are not easily seen, rather, parts of cells and atrophied epidermal cells are seen. It is common for epidermal cells to shrink in the adult stage of hemimetabolous and holometabolous insects.

There are also perikarya within or associated with this layer that belong to afferent neurones associated with cuticular sense organs. Considering the large number of socketed hairs on the legs, it is suprising that more neurones are not visible (including dendrites in channels in cuticle).

Another cell-type commonly identified in the epidermal cell-layer is a gland cell. This cell-type

is easily indentifiable because of its large size (approximately 6–10 μm) and its characteristic duct (see figure 4.4). Density of gland cells in areas without basiconic sensilla is 2 · 9 cells per 100μm (n=10; s=2 · 1). Moreover, if cuticle is examined closely, pore canals of less than 1μm are discernable; the size of these canals is consistent with the size of ducts within the cell bodies (see figure 4.5). These canals can also be observed from the cuticular surface using an SEM, after having cleaned the specimen, removing cuticular wax (see figure 4.6); and are discussed in more detail in chapter 2. Gland cells of this type are characteristic of Gerromorpha (personal communication, BS Heming). It is highly likely that these gland cells secrete substances conducive to maintaining hydrophobic qualities of cuticle and the hair-layers.

4.3.3 Mechanosensitive Structures

Figure 4.7 illustrates a dendritic channel in a socketed hair the actual dendrite is not distinguishable. This demonstrates that this hair-type is innervated.

Two types of campaniform sensilla were identified. The first is that of the smaller type found ubiquitously on all three leg-pairs (see figure 4.7). The second campaniform sensillum is that of the spindle-shaped sensillum located on the trochanter of all three leg-pairs in the 4+3+3+3 arrangement (see chapter 2). This sensillum type is a character Andersen (1982) uses in defining the groundplan of Gerromorpha. Figure 4.9 shows well the dome as well as the receptor cavity. In fact, the receptor cavity is quite large (about 60μm wide in this section). Possibly three dendrites may be identified within this section (only one is shown in figure). This suggests that the subcuticular space below these sensilla is one large receptor cavity (i.e., that one receptor cavity houses neurones of either 3, 4, 6 (3+3), or 7 (4+3) campaniform sensilla; and the three dendrites correspond to three campaniform sensilla. I believe that there is one cavity for three sensilla (or four in the case of the 'four group'). This makes sense when one considers the 4+3 or 3+3 group of sensilla probably function as an integrated or unified unit.

4.3.4 Basiconic Sensilla

Figure 4.8 shows a basiconic sensillum with its receptor cavity going through the cuticle. A closer view of the cavity reveals three dendrites (only two of which are visible). Figure 4.10 is a photograph of another basiconic sensillum underneath which is an enlarged epidermal cell-layer. Presence of three dendrites, and an enlarged epidermal cell-layer are strong evidence for a multi-innervated sense organ. Figure 4.11 is a composite based on many sections and data from the cell counts.

The exact number of neurones innervating an individual sensillum is not clear. Sectioning of 40 blocks did not produce a section (or series of sections) showing all dendrites clearly, nor did it produce a section (or series of sections) with definitive perikarya.

It was possible to reach some conclusions about the number of neurones innervating a sensillum by carrying out cell counts underneath sensilla, and comparing them to counts taken from a basiconic sensillum-free area. (It should again be mentioned that not all cells are easily countable in the epidermal cell-layer because of pinocytosis).

The average number of cells (minus gland cells) under a basiconic sensillum is 42 · 5 cells per 90μm (s=6 · 6; n=10). The average number of cells (minus gland cells) in a basiconic sensillum-free area is 24 · 8 cells per 90 μm (s=6 · 2; n=10). This gives a difference in cell numbers of 17 · 7 cells per 90μm (s=7 · 8; n=10). The difference is significant (U=3; n_1=10, n_1=10; $p<0 \cdot 002$).

There are, then, an average of 18 more cells beneath a basiconic sensillum in a 4–5 μm section. I suggest these cells are intimately involved with the basiconic sensillum. A minimum of three cells could be accessory (one inner-sheath cell; one outer-sheath cell; and one intermediate-sheath cell) or a maximum of six cells (additional sheath cells) (Zacharuk, 1985). It is also possible that one cell may be a mechanosensitive neurone associated with the basiconic

sensillum (Zacharuk, 1985). The remaining 11 to 15 neurones could be chemosensitive.

This is a reasonable number of neurones to be associated with a chemosensitive sense organ of this type (Chapman, 1982). There may in fact be more neurones, for it is likely that not all cells were contained within one 5μm section

While the exact number of cells is not known, evidence is sufficient to conclude that the basiconic sensilla are multi-innervated. I suggest they serve a chemosensitive function.

4.4 Conclusion

Histological techniques used in this chapter show that the cuticle in *Gerris remigis* (as well as other water-striders) is very thick and heavily sclerotised. This is a characteristic that contributes to this insect's robustness. It also demonstrates the histological and structural difficulties involved in histological studies of these animals. In this case, techniques appropriate to obtain the objectives were limited; it would have been beneficial to have complete serial sections at a greater thickness (7–10μm). This step had to be omitted because of embedding difficulties, and incomplete serial semithin sections had to suffice.

Much information has been gained from this work. Leg cuticle is quite thick and strong. Gland cells — characteristic of the Gerromorpha — can be related to cuticular pores and glandular material found in SEM analyses (see chapter 2). In the spindle-shaped campaniform sensilla located on the trochanters, the receptor cavities are perhaps enlarged into one to accomodate from three to seven dendrites (related to the three to seven campaniform sensilla).

However, the main purpose of this work was to find evidence to support the theory that basiconic sensilla found on *Gerris remigis* male's mesothoracic and metathoracic trochanters and femora are multi-innervated chemosensitive sensilla as opposed to being specialised mechanosensitive organs or even non-innervated cuticular structures. This work does provide

satisfactory evidence that these sensilla are multi-innervated. The strongest evidence is the three dendrites seen in the receptor cavity in one section. Multi-innervation is further suggested by a significantly greater number of cells within the epidermal cell-layer under a basiconic sensillum (18 cells per 90μm) compared to a basiconic sensillum free-area.

My conclusion that these sensilla are, indeed, chemosensitive is based on the following: (i) In general, Type I mechanosensitive organs are innervated by one neurone while chemosensitive organs are multi-innervated. (ii) SEM analysis of the cuticular surface of these basiconic sensilla reveals many apparent pores (presence of a pore or pores is usually a necessary criterion for claiming chemosensitive function, although there are exceptions)(Zacharuk, 1985). (iii) There is some evidence of a pheromone-mediated mating system in *Gerris remigis* (see chapters 1 and 5).

It is unfortunate that an exact number of neurones associated with a basiconic sensillum could not be determined; technical limits and cell size contributed to this. 40 blocks were sectioned, yet no sections provided a complete picture of the structure of this sensillum type. This sugggests that TEM work be the next step in determining the structure of this basiconic sensillum; although individual sections would contain fewer structures, they would provide more detail.

Figures 4.1 to 4.6. Semithin sections and SEM photomicrographs of metathoracic and mesothoracic trochanters and femora of *Gerris remigis* males. C, cuticle; DC, dorsal compartment; E, epidermal cell; ECL, epidermal cell layer; GC & GD, gland cell & duct; M, muscle; N, nerve; PC, pore canal.

Figure 4.1. Cross-section at joint. Scale bar = 100μm.

Figure 4.2. Longitudinal-section at joint. Scale bar = 100μm.

Figure 4.3. Cuticle and epidermal cell-layer. Longitudinal section. Scale bar = 20μm.

Figure 4.4. Gland cell in epidermal cell-layer. Scale bar = 10μm.

Figure 4.5. Cuticle showing pores. Scale bar = 20μm.

Figure 4.6. SEM photomicrograph of these cuticular pores. Scale bar = 10μm.

DC
T
T
T
N
1
ECL
C
N
T
2
CC
GD
3
4
PC
PC
5

Figures 4.7 to 4.10. Semithin sections of metathoracic and mesothoracic trochanters and femora of *Gerris remigis* males. C, cuticle; CS, campaniform sensillum; CSD, campaniform sensilla dome; D, dendrite; DC, dendritic channel; ECL, epidermal cell layer; H, hair; M, muscle; N, nerve; P, peg (sensillum); RC, receptor cavity;

Figure 4.7. Socketed hair and campaniform sensillum. Scale bar = 20μm.

Figure 4.8. Basiconic sensillum and receptor cavity. Scale bar = 10μm.

Figure 4.9. Dome and receptor cavity of a campaniform sensillum. Scale bar = 20μm.

Figure 4.10. Basiconic sensillum and enlarged epidermal cell-layer. Scale bar = 20μm.

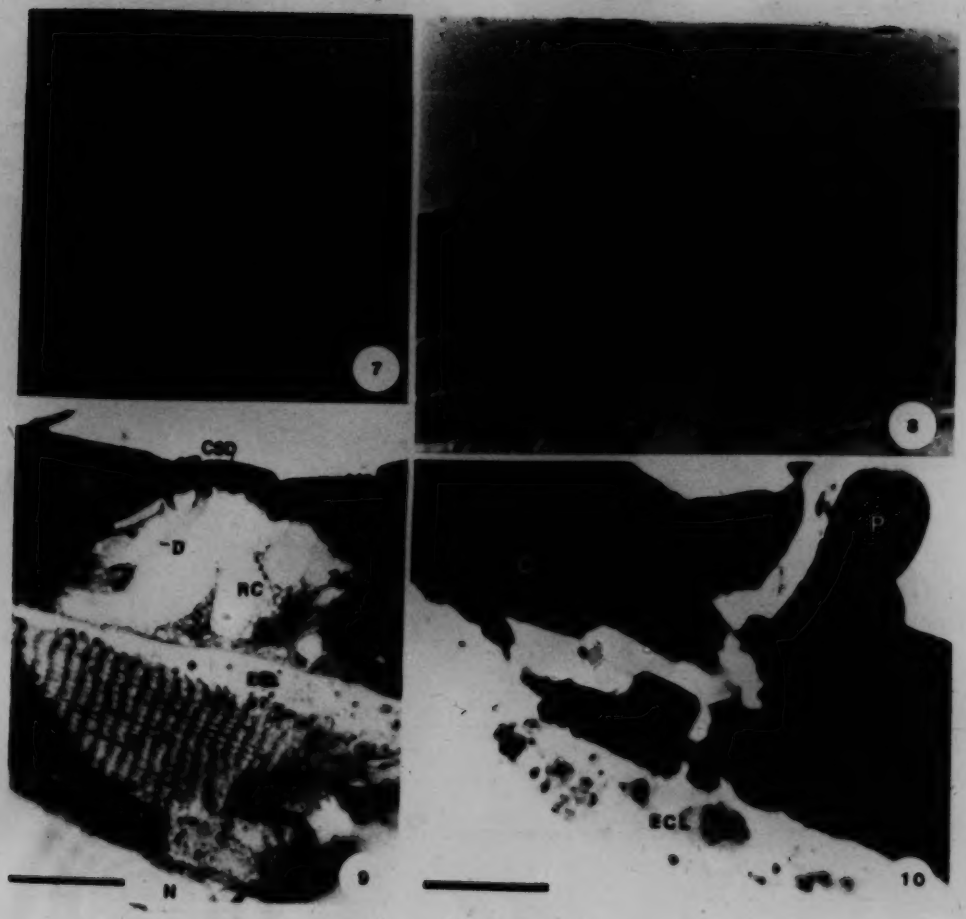
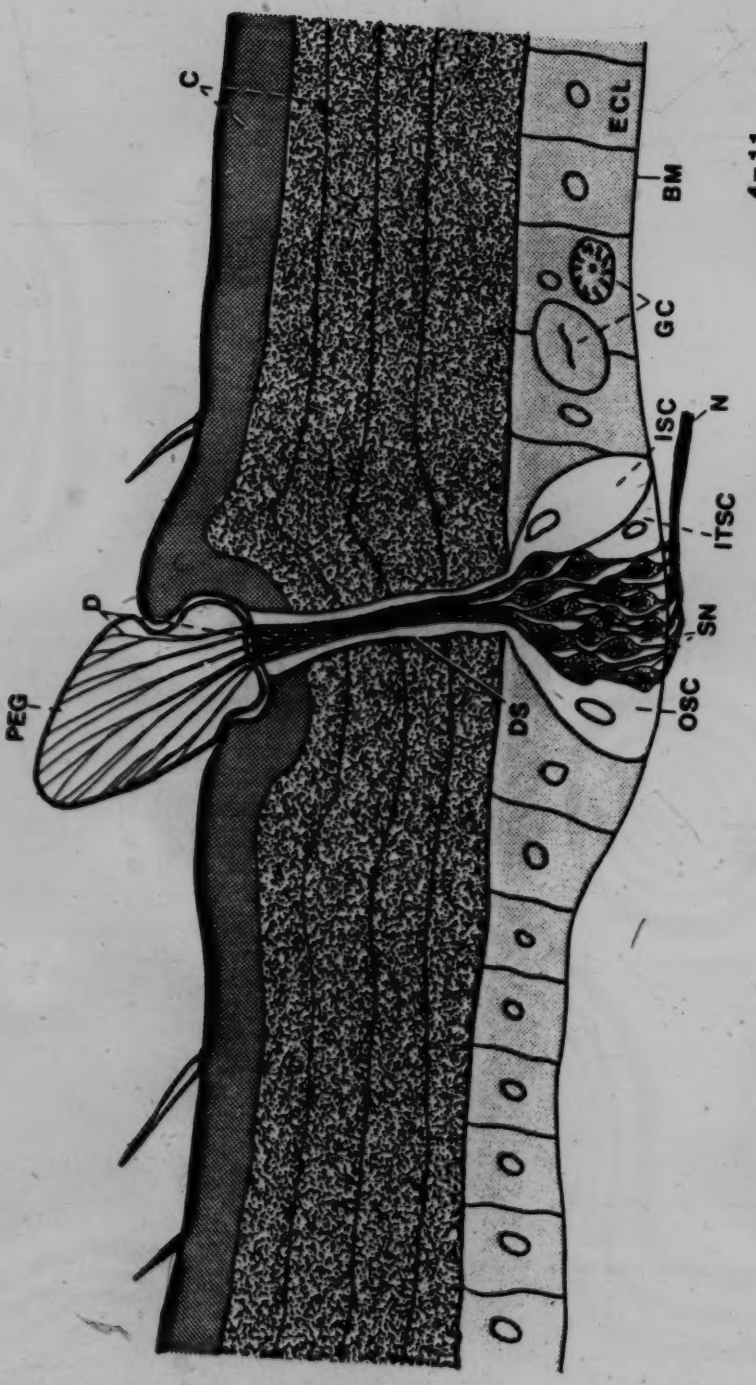

7
8
CSO
D
RC
N
9
P
ECL
10

Figure 4.11. Suggested structure of the basiconic sensillum sense organ. Reconstructed from SEM photomicrographs, many sections, and from cell count data. BM, basement membrane; C, cuticle; D, dendrite; DS, dendritic sheath; ECL, epidermal cell layer; GC, gland cell; ISC, inner-sheath cell; ITSC, intermediate-sheath cell; N, nerve; OSC, outer-sheath cell; PEG, sensillum; SN, sensory neurones.



4-11

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5. A Study of Mating Behaviour in *Gerris remigis*

5.1 Introduction

In chapter 2, I described a basiconic sensillum located on male mesothoracic and metathoracic femora and trochanters of *Gerris remigis*. Because of the shape of the sensillum, sexual dimorphism (females lack these sensilla), and absence in nymphs, I hypothesised that this type of sensillum is involved in mating behaviour, detecting a low volatile contact pheromone produced by the female.

The sensillum is cone-shaped, has a base width of approximately 20 micrometres, a length of approximately 25-35 micrometres, and is situated on a dome-like base. The sensillum is slightly striated and contains small pores. From histological work, it appears that the sensilla are innervated with several neurones. Because of the shape, pores, and innervation, the sensillum probably has a chemosensitive function. It is also possible that the sensillum is mechanosensitive in function, i.e., a specialised, multineuronal campaniform sensillum, but this is not likely.

In order to further elucidate the function of these sensilla, I carried out a series of behavioural observations and experiments on mating behaviour.

The majority of work that has been done on mating behaviour in Gerridae has included three groups, the *Limoporus rufoscutellatus* group; *Gerris (Aquarius)*, the subgenus to which *Gerris remigis* belongs; and *Rhagadotarsus kraepelini*, a more derived gerrid. Most of the *Limnoporus rufoscutellatus* work deals with spacing and courtship signalling, territoriality, and oviposition (Spence & Wilcox, 1986; Wilcox & Spence, 1986; Vepsäläinen & Nummelin, 1985). *Rhagadotarsus kraepelini* studies deal with signalling systems in mating (Wilcox, 1972).

Wilcox (1979) has analysed signalling in *Gerris remigis*. Males use presence or absence of high frequency signals (85-90 Hz) to identify other males; only males signal and only males show a behavioural response to high frequency signals. *Gerris remigis* is not territorial nor do males signal from a signal site. Through blind-fold experiments, Wilcox has shown that visual cues are not necessary for a male to locate and mount a female. He has also shown that chemical cues are (if existent) not necessary for mating. Wilcox's research shows that signalling plays a major rôle in mating behaviour in *Gerris remigis*. However, his research is oriented towards mechanical signalling systems. Little work has been done in a context different from this.

The purpose of this work was to study and describe in detail a mating sequence in *Gerris remigis*. This in turn can be used as a reference to test the hypothesis that a pheromone-mediated mating system is present in this species; and to find evidence for the function of the basiconic sensilla on the male mesothoracic and metathoracic femora and trochanters.

5.2 Materials and Methods

Gerris remigis Say males and females, and *Limnoporus dissortis* (Latreille) females were used. Animals used in these various observations and experiments were collected from George Lake Field Station (Department of Entomology, The University of Alberta) located 16 kilometres west of Busby, Alberta; Little Hornbeck Creek, located 20 kilometres west of Edson, Alberta; and Whitemud Creek in Edmonton, Alberta. (Voucher specimens are deposited in the University of Alberta Strickland Museum.) Some were overwintered and others collected in late spring to early summer. They were maintained in an incubation room with a constant temperature of 18°C in plastic containers and fed on vestigial-winged *Drosophila*.

Mating Behaviour Observations: Nineteen virgin males and nineteen virgin females of *Gerris remigis* were used in the study. The observation chamber was a round plastic container with a

surface area of 491 square centimetres. Behind the container was placed a neutral-coloured screen. Each event was video-taped from a distance of 2 · 5 metres. Experiments were carried out between 1300 and 1600 hours in a room of a constant 24°C and 46% relative humidity.

A female was introduced into the chamber and given two minutes to adapt. The male was then introduced and the observation period began. Half of the observations were carried out until the male and female decoupled; the remainder were terminated five minutes after time zero. These observations were used to describe a complete mating sequence for *Gerris remigis* in order to note any behaviours that are suggest a contact pheromone-mediated mating system. This also served as a control or base from which to work when analysing data from experiments.

Other Observations: Six virgin females and ten virgin males were kept isolated for 24 hours and then were put with another of the same sex in an observation chamber. The set-up and recording was the same as that for the mating behaviour observations. Recording was stopped at two minutes with females and five minutes with males. The purpose of these observations were: (i) to observe similarities and differences in male-male and female-female interactions compared with male-female interactions; and (ii) to gain further data on approaches and contacts.

Five virgin male *Gerris remigis* and five virgin female *Limnoporus dissortis* were kept isolated for at least 24 hours prior to observation period. Males were introduced to females in the same manner as described above. Recording period was five minutes. The purpose of this observation was to observe similarities and differences as compared to that of interactions of *Gerris remigis* females. Also, to note approaches, orientation, and reactions to females by males.

Observations of mating in the dark were carried out with ten virgin males and ten virgin females of *Gerris remigis*. Video-recording was not possible, so observations were recorded with a stop-watch (a dark-room lamp was used to allow for observing and timing). The purpose of

this observation was to confirm findings (from literature) that visual cues are not necessary for mating. And, to compare with mating behaviour in light.

Antennaectomized Males: Antennae of nine virgin males were cut off and the animals were left to rest for 24 hours. They were introduced to a female in the observation chamber. Each mating was recorded on video-tape. The purpose was to note if mating was impaired or different from that of the control group. A difference would indicate possible chemosensitve structures on the antennae that are used in mating behaviour.

Painted Receptors: Mesothoracic and metathoracic trochanters and femora of nine virgin males were painted with enamel paint and left for 24 hours before experiments. Males were introduced to females in the chamber as previously described. The behaviour was video-taped. The purpose was to see if blocking the receptors on the mesothoracic and metathoracic trochanters and femora would significantly alter mating behaviour.

Pheromone Model Experiments:

Extractions. Extractions were made by freezing females at -18°C for one hour. They were then slurreyed one and a half minutes (in each beaker) in a series of: acetone–acetone–methanol–methanol (modified from Klinjstra, 1985). Acetone washes were combined and methanol washes were combined. Each wash was evaporated with a flash evaporator Büchi Rotovapor – RE/B. Residue of each wash was weighed and then acetone and methanol were added to respective residues to standardize concentrations. Concentrations are expressed as gerrid-equivalents per millimetre of solvent (geq/ml). The solutions were kept refrigerated.

Models. Three types of models were used: (i) freshly killed females (killed by freezing one to two hours prior to experiments). (ii) 'old' models were made by freezing females, thawing them, and placing them over a small strip of plasticine on a styrofoam board; the legs

were arranged and pinned in a way that allowed a natural looking posture on the water. They were left to dry for one to two weeks. 0 · 2 ml of acetone and methanol were applied to the female's abdomen and thorax immediately prior to experiments. (iii) 'washed' models were prepared in the same manner as the old models and then 0 · 2 millimetres of acetone wash and methanol wash were applied to the thorax and abdomen to give a concentration of 1 · 04 gerrid equivalents.

Experiments. The same set-up, involving a container and screen, was used as with other observations. A male was presented with the three types of models with a five minute period between each model. Presentation of models was ordered in a 3! fashion (i.e., 3,2,1; 1,2,3; etc.). Six males were used for a set, and the set was replicated three times, so a total of eighteen males were used in the experimnet. An encounter with a model was allowed to continue for five minutes and then it was terminated. Scoring consisted of: failed attempt, copulatory, feeding, no interaction.

Statistics: Statistical analysis was based on both parametric and non-parametric statistics using Sokal & Rohlf (1981) and Siegal (1956) respectively. Analysis was done using the statistical package MIDAS.

5.3 Results and Discussion

Mating Behaviour: Figure 5.1 shows an ethogram of the mating behaviour sequence in *Gerris remigis*. After the male was introduced, there were some male chases as well as some female chases. (One-third of the time the female appeared to chase.) Contact between male and female occurred, on average, three to four times before copulation, the female initiating the majority of contacts (see table 5.1).

When the male approached the female for mounting, he approached her from the side (rarely

from behind). At this point three of 19 observations showed the male signalling. Since high frequency signalling is not easily observed, it is difficult to say whether signalling occurs in all cases or in just a few instances. (The actual waves, because of their small size, cannot be observed, but a specific postural behaviour can be.)

When the male's body axis was perpindicular to the female's, he grabbed the female's thorax and mesothoracic legs with his prothoracic legs. Within a second, the male moved on top of the female so that his body axis was parallel with the female's, while simultaneously starting to protract his aedoeagus. Within another second, the male inserted his aedoeagus into the female. Also, he rubbed his mesothoracic femora against the female's abdomen. The rubbing act consisted of several, very quick motions not lasting more than one second. Frequently there were leg rubbing bursts — again, not lasting more than one second in duration and continuing throughout the insertion period (especially if the female became very active). (The average number of leg rubbing bursts was $2 \cdot 4 \pm 1 \cdot 1$.) While the male was inserted, he rode around on the female's back. Both male and female groomed themselves, the female grooming more actively. During this period the female often struggled, but sometimes remained passive. In half the observations there was some degree of struggling on the part of the female.

Separation appeared to be initiated by the female in most cases. This happened within a few seconds to nearly an hour after mating. After decoupling, they either resumed chasing, in effect, started the cycle over, or they remained apart with no futher chases. The former usually happened if separation occurred within five minutes of insertion. Based on a sample of ten matings the average mating time was 25 minutes ($s=12$ minutes, see table 5.1).

The fact that both the male and female will chase indicates a readiness to mate. While *Rhagadotarsus kraepelini*, a water-strider from Australia, has a signalling system where the female produces courtship signals, as well as the male, and actively makes contact with him (Wilcox, 1972); in *Limnoporus*, males seem to be the only ones who are aggressive in making contact (Spence & Wilcox, 1986; Wilcox & Spence, 1986). Since *Limnoporus* is a sister genus to

the *Gerris-Neogerris* group, it would seem that mating behaviour would be more similar among these groups than with the more derived *Rhagadotarsus* . In this respect, it is curious that the female in *Gerris remigis* is aggressive in making contact with the male.

Another point worth noting in comparison to *Limnoporus* is the readiness to mate. *Gerris remigis* will often mate within seconds of introduction. Moreover, they are not easily disturbed; whereas in *Limnoporus*, mating can be capricious, taking a long time for the male to mount, both sexes being sensitive to movement and noise.

The orientation behaviour of the male prior to making contact with the female is interesting. It allows the male to grab the female securely and to be in a proper position for protraction of aedoeagus. As mentioned above, the absence of high frequency signalling, in many observations, could just be an observational artifact. There is no doubt that high frequency signalling plays an integral rôle in mating behaviour in the field, but how necessary and how frequently it is used under laboratory conditions is not known. The observation chamber being rather limited in size (491 square centimetres), perhaps visual cues (and perhaps chemical cues) are sufficient for sex recognition.

Male leg rubbing on the female abdomen is suggestive of a chemosensitive function with a contact pheromone being secreted on the abdomen of the female and the male sensing the pheromone by receptors (associated with the basiconic sensilla) on the male mesothoracic and metathoracic trochanters and femora. The fact that there are more basiconic sensilla on the mesothoracic leg, which is rubbed against the female's abdomen, is consistent with this hypothesis.

If this leg rubbing act is significant in a function other than just described above, what is it? One possibility is that this act (through chemical stimulation or rhythm) is a signal to the female, for example, a calming aid. However, this is a regular part of a mating sequence and is observed even when the female is quiescent and does not resist mounting.

Other Observations: When females were observed together there was very little interaction. They groomed themselves and would occasionally contact each other when moving around ($x = 3$ contacts, $s = 1$). When contact took place, there was no evasive action or struggling. They did not chase (as they did on occasion when placed with a male).

When males were observed together, there was considerably more activity than observed when females were together. There was an average of 13 contacts per pair ($s=4 \cdot 9$) almost all of which were a direct result of chasing. Attempts to mount generally followed chasing, with some orientation as when with a female. Simultaneously there was a partial bending of the aedoeagus (except for one observation), at this point, the bottom male would raise up and signal. This was followed by struggling and an immediate break up. This behaviour would be repeated several times throughout the five minute observation period. It is important to note that males were much more aggressive than females. There was chasing, mounting attempts with proper orientation, vibrations, and struggling; however, no leg rubbing was ever observed. It might also be mentioned that in two observations there was actual aggressive fighting lasting several seconds, this was different than just avoidence struggling.

When males were introduced into the chamber with *Limnoporus dissortis* females, a species which is the approximate size of *Gerris remigis*, in four out of the five observations, the male chased the female. When contact was made (usually by a rear approach) the male discontinued contact. He sometimes began chasing again with intermittent grooming. The female avoided the male.

The purpose of making these observations was to note differences and similarities in interactions of males and females (as well as a species of similar size) paying particular attention to approach and orientation. Because of their apparent lack of response to signalling and their passive rôle in coupling (Wilcox, 1979), it is not suprising that when females are put together, they show little response to each other. At the same time, it is interesting that the

females will chase a male. This indicates that the females may be able to distinguish sex, probably by visual cues and maybe other cues. It appears that females are more active and more aggressive when placed with a conspecific male.

Males chased females, other males, and *Limnoporus dissortis* females in very much the same way. This indicates that visual cues (using body size as a guide) are used in recognising potential mates. When males were introduced to other males they even oriented, grasped, and started to mount (including beginning protraction of aedoeagus), but stopped short of leg rubbing because the bottom male had begun signalling by this time. When a male was introduced to a *Limnoporus dissortis* female, after chasing, the behaviour was quite different in that orientation was not from the side (as is with both male and female *Gerris remigis*), but from the rear. Once contact was established, the male moved away from the female. The difference in approach and contact between *Gerris remigis* males and females and *Limnoporus dissortis* females indicates that there is some sort of recognition immediately prior to, and at contact. This recognition could be from sight and or some type of chemical cue. If it is a chemical cue, it seems to be present in both males and females of *Gerris remigis*.

Mating Behaviour in Dark: Qualitatively mating behaviour in dark was consistent with observations made in light. Because of the inability to video-tape, the only measurement taken was time from introduction to leg rubbing. (This time period was chosen because it could be measured accurately.) There was no significant difference in the time between introduction to leg rubbing with dark and light observations ($U=71$; $df=19,10$; $p=0\cdot27$)(see table 5.3).

This finding is in agreement with that of Wilcox's (1979), where his experiments were done by blind-folding the males. He showed that visual cues are not necessary for male recognition and that recognition is made by absence or presence of high frequency signals. As mentioned above, I did not see high frequency signals in all observations, but, it seems that from the dark room mating observations, *Gerris remigis* does not need visual cues for mating. (I make an assumption that *Gerris remigis* is unable to detect red light frequency of approximately 625 –

700 nanometres.)

This is intriguing, because, while it is shown that visual cues are unecessary in the mating process, the observations of mating in light indicate a very strong reliance on visual cues. The fact that chasing, orientation, and size discrimination occur in the way they do point towards visual cues. Even if there are chemical cues, it is difficult to explain the side orientation and approach demonstrated so consistently in the observations. Perhaps *Gerris remigis* does rely on visual cues, but when these cues are affected, *Gerris remigis* is adaptable enough to use other cues to succeed in mate recognition.

Antennaectomized Males: The same behavioural acts measured in the control group (mating behaviour observations) were measured in antennaectomized males. U tests were performed on approach ($U=77$; $df=19,9$), aedoeagus bending ($U=77$; $df=19,9$), and leg rubbing ($U=78$; $df=19,9$)(see table 5.2). At $p> 0 \cdot 025$, no significant difference was found between the two groups.

It would not have been unreasonable that the males would be lethargic and behaviour somewhat modified because of manipulation of the insect. Despite considerable bleeding, males recovered quite well from the antennectomies. The mating sequence was consistent with that of the control (see table 5.2). This shows that: (i) antennae are not necessary, and probably not extensively involved, in mating; and (ii) if a pheromone system is involved in mating behaviour of *Gerris remigis*, receptors for the pheromone are not located on antennae.

Painted receptors: By painting the mesothoracic and metathoracic femora and trochanters of males, mating behaviour was significantly altered. There was a significant difference from time of introduction to mating ($U=43$; $df=19,9$; $p<0 \cdot 025$)(see table 5.2). There was also a significant difference in the male's approach time ($x^2=9 \cdot 85$; $df=1$; $0 \cdot 05$). There was no significant difference in mating frequency ($x^2=1 \cdot 41$; $df=1$; $0 \cdot 05$).

Males with painted legs were fairly quiescent and did little chasing. They appeared to have

difficulty in judging direction as well as in orientation after contact with the female was made. Four of the nine males mounted in a manner different from that observed in the control group. Usually mounting took place so that the male's head was at the abdomen of the female. Eventually orientation would be righted and mating took place. Leg rubbing generally took place, but was slower and not as frequent.

Since nine out of eleven males mated, the necessity of these putative chemosensitive sensilla in actual mating is questionable. The time before mounting and orientation, however, were affected. But, looking at the behaviour of these males in a qualitative manner, I believe differences in time (and possibly orientation) are a direct resut of the physical presence of the paint.

Prior to the experiment, painted males spent much time on styrofoam platforms in the container where the Gerrids were kept. Their body positioning was also considerably lower to the water surface when with females, and feeding. (Normally the body is positioned well above water when with females, mating, and in other situations.) These observations in conjunction with the general lethargy of the striders suggest a physical hindrance caused by the paint. (Joints on the legs were functional.)

The poor judgement of distance and orientation are more difficult to explain in terms of the paint. Perhaps the basiconic sensilla are used in helping to judge distance and to orient to the female, but this is unlikely. The lethargy and lowered body positioning are probably caused by the paint covering up the hydrofuge hairs on the legs making it difficult to move with agility and maintain proper body positioning. Because of this, I am reluctant to place much reliance on this. I do not believe anything can be concluded from this experiment with any certainty. However, the lack of proper orientation and poor judgement are interesting and it is possible that the sensilla have some influence on these behaviours.

Pheromone Model Experiments: The majority of males fed upon or ignored all three types of

models. Three males attempted copulation with a fresh female, and there was one failed attempt with a washed model. Statistically, behaviour was independent of models ($x^2 = 5 \cdot 06$; $df = 8$; $0 \cdot 05$).

I hypothesised that males would mate with a freshly killed female, with a washed old female, but not with an old female. And, that there would be a certain amount of feeding and non-interaction. This is based on the assumption that females have a chemical present on their cuticle that acts as a pheromone; that in freshly killed females this chemical would still be present in quantities strong enough to attract males; and that a washed female would have the chemical applied to it from extractions. Moreover, in field experiments using untreated models with *Limnoporus dissortis*, males were receptive to freshly killed models (2–3 hours), but fed on day old models (personal communication JR Spence).

There are four possibilities that can explain these results. (i) There is no pheromone system and the sensilla have another function. Or if there is a pheromone system, it works in conjunction with other systems where the female, for example, must be alive so that the other systems are complete. (ii) The freshly killed female models, because they are not dried, do not maintain optimal position on the water — they are lower on the water. This could explain why males fed on freshly killed females, suggesting that body positioning is an important cue. If this is the case, then why were washed female models — which had very good leg positioning — fed on by males? (iii) It is possible that the extractions were not strong enough in washed female models ($1 \cdot 04$ geq) in order to compensate for the models being dead. It is also possible that the extraction procedure did not extract the pheromone. In either case, this could lead to feeding rather than mating. (iv) The pheromone could be extremely volatile, and it may be necessary that it be produced continuously by the female.

The first and fourth possibilities explain most completely the results of this experiment, or the second and third combined. I believe the fourth possibility is least likely because it would not be advantageous to have such a short-lived pheromone. I believe the most parsimoneous and likely

possibility is the first. A different experimental design — including stronger concentration of extract, different method of preparing freshly killed models, and more repetitions — might lend insight into which of the possibilities is correct.

If their is no pheromone system involved in mating behaviour of *Gerris remigis* (which the results suggest), then the function of the basiconic sensilla needs to be reconsidered.

5.4 Conclusion

The evidence in favour of a contact pheromone-mediated mating system is the behavioural act of leg rubbing by the male on the females abdomen, and the ability to mate in the dark. While it was mentioned earlier that the leg rubbing act could be a mechanical stimulus to calm the female, it is doubtful this is its function since it is performed regardless of whether the female is passive or active. Results from mating in the dark observations do not necessarily corroborate the above theory, however, they show that visual cues are unimportant. Outside of visual cues, there are possibly chemosensory and other mechanosensory cues. It is possible that high frequency signalling was involved, and, it is possible that the males could have been attracted to a pheromone. It is difficult to say which of the two cues were used, but, it is obvious that both males and females could note presence of another animal in the chamber by sensing waves created by the other.

Evidence that does not favour a contact pheromone-mediated mating system derives from the model experiments and experiments where the receptors were blocked. Males did not mate with the models (except for three males that attempted copulation with freshly killed female models). If females produce a pheromone, and even if the extraction procedure did not extract the pheromone, a larger number of males should have attempted copulation with the freshly killed models — it is unlikely that the pheromone would dissipate that quickly.

Application of paint on the legs of males may have caused abnormal behaviour (such as poor

orientation and lethargy), it did not, however, arrest mating. With paint blocking the presumed receptors, males still mounted, rubbed their legs on the females abdomen, and inserted.

Results from the model experiments seem to suggest that there is no pheromone-mediated mating system. I do not believe the results are conclusive. The leg rubbing act, the chemosensitive sensilla (and sexual dimorphism), are strong indicators of a pheromone involvement. Another experimental design and a different extraction technique may provide different results.

Figure 5.1. Ethogram of mating sequence in *Gerris remigis*.

ETHOGRAM

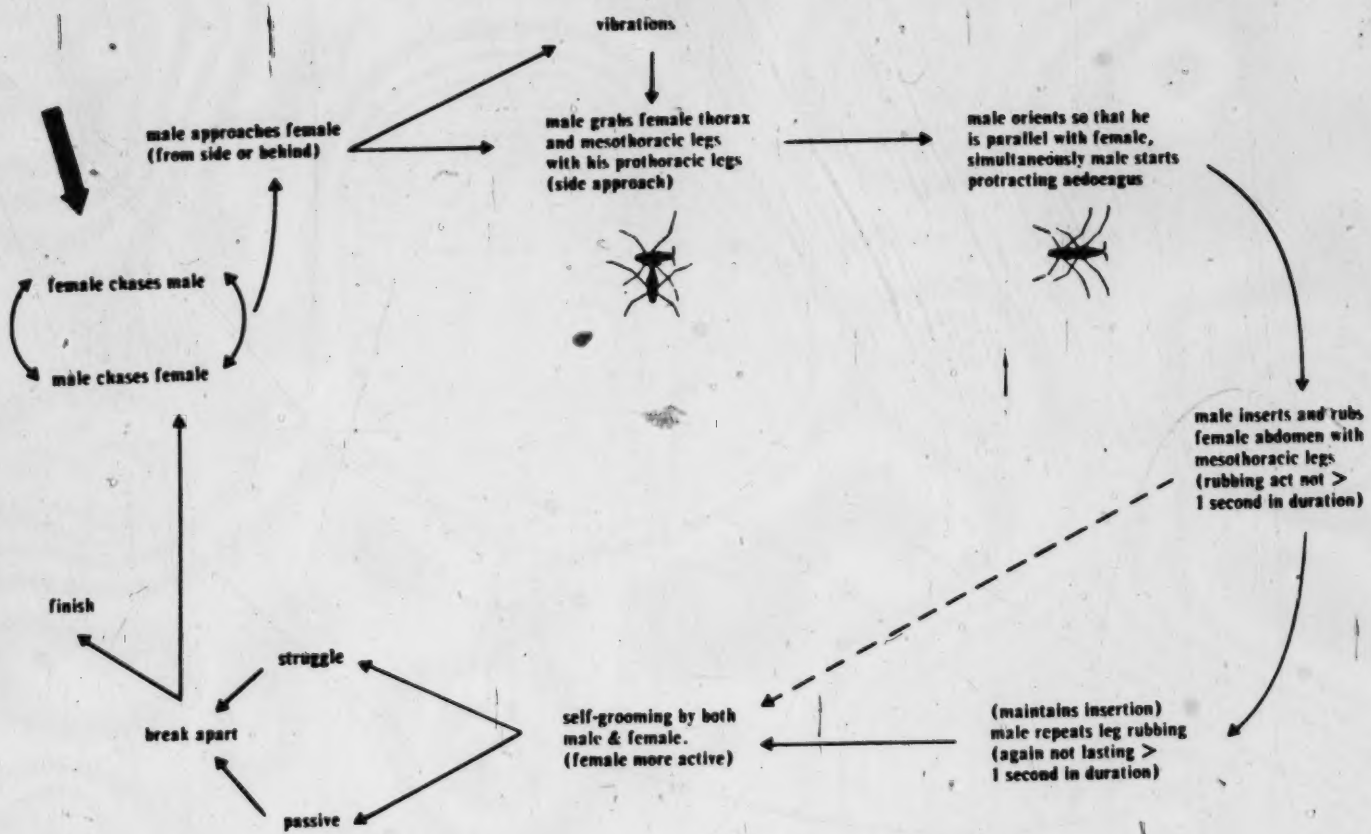
vibrations
male approaches female
(from side or behind)
male grabs female thorax
and mesothoracic legs
with his prothoracic legs
(side approach)
male orients so that he
is parallel with female,
simultaneously male starts
protracting aedoeagus
female chases male
male chases female
male inserts and rubs
female abdomen with
mesothoracic legs
(rubbing act not >
1 second in duration)
finish
struggle
break apart
passive
self-grooming by both
male & female.
(female more active)
(maintains insertion)
male repeats leg rubbing
(again not lasting >
1 second in duration)

Table 5.1. Data of mating sequence of Gerris remigis.

behaviour (n=19)	time (sec)	frequency	trials out of 19	seconds maximum	seconds minimum
number of contacts before mating:					
males and females		3.4±5.3			
female initiated		2.2±5.0			
male initiated		1.3±1.8			
female chasing			6		
male approach (time 0 until contact with prothoracic legs)	81.8±108.2			443.6	4.8
time 0 until aedoegus bending	82.8±107.9			443.7	4.9
time 0 until leg rubbing	84.5±107.9			444.4	6.7
leg rubbing bursts		2.4±1.1			
time from approach to aedoegus bending	1.0±0.8				
time from aedoegus bending to leg rubbing	1.7±1.7				
grooming			19		
struggling females			9		
signalling (vibrations)			3		
mating length (n=10)	25min 20sec (±12min)				

Table 5.2. Data of mating sequence of Gerris remigis with antennaectomized males.

behaviour (n=9)	time (sec)	frequency	trials out of 9	seconds maximum	seconds minimum
number of contacts before mating:					
males and females		1.1±0.6			
female initiated		1.0±0			
male initiated		1.4±0.6			
female chasing			3		
male approach (time 0 until contact with prothoracic legs)	49.4±57.7			192.9	4.9
time 0 until aedoegus bending	51.9±59.1			197.2	5.4
time 0 until leg rubbing	54.1±59.2			197.4	8.0
leg rubbing bursts		3.2±1.6			
time from approach to aedoegus bending	2.5±2.5				
time from aedoegus bending to leg rubbing	2.3±2.9				
grooming			9		
struggling females			1		
signalling (vibrations)			0		

Table 5.3. Data of mating sequence of Gerris remigis with blocked receptors and in dark.

behaviour	n	time (sec)	seconds maximum	minimum
blocked receptors time 0 to approach	9	377.9±428.1	1092	20
mating in dark time 0 to approach	10	108.0±105.8	382	14

5.5 Literature Cited

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6. Conclusion

In the process of carrying out an SEM study of the legs of *Gerris remigis*, I described basiconic sensilla on male mesothoracic and metathoracic trochanters and femora. Because of sexual dimorphism and the suggestive shape of the sensillum, I hypothesised there is a pheromone-mediated mating system in which the basiconic sensilla function in detecting a pheromone. The study focuses are the basiconic sensilla, their structure, and their putative function in mating behaviour.

It was important to understand what the distribution of basiconic sensilla are within the family; if they are specific for *Gerris remigis*, or found in various groups. It appears that presence of basiconic sensilla was independently derived twice. Once in *Gerris remigis* and once in the *Limnoporus rufoscutellatus* group (subfamily Gerrinae). In the *Limnoporus rufoscutellatus* group, however, the sensilla are found only on mesothoracic legs, and in both sexes. While it is assumed they are homologous structures with those of *Gerris remigis*, because they are present in both sexes raises the question of whether they are analogous in function. I believe they are analogous in function; my main reason supporting this is that mating behaviour (and behaviour in general) is rather similar in both species.

Histological investigations revealed that the basiconic sensillum is, indeed, chemosensitive. There appear to be 11 - 15 neurones innervating each sensillum. Moreover, presence of many apparent pores on the sensillum surface indicated that the sensillum is a contact chemosensillum. (This lends support to the theory of a phereomone-mediated mating system hypothesised — i.e., a contact pheromone is involved.)

After determining the distribution, and the structure and physiological function, the next step was to determine the behavioural function of these sensilla in *Gerris remigis* (namely, are they involved in mating behaviour). Electrophysiological studies in which the receptors of the sensillum are excited by a chemical derived from a female, and behavioural studies whereby a

pheromone-mediated mating system can be demonstrated, are necessary criteria in order to determine if the behavioural function of the sensilla is part of the mating process. Behavioural experiments were carried-out. Some of the results corroborated a pheromone-mediated mating system and some did not.

All evidence, attained thus-far, that support a pheromone-mediated mating system in *Gerris remigis* are: (i) Basiconic sensilla are not seen in nymphs, nor are they seen in females. (ii) The leg rubbing act performed during mating is an opportune time for the receptors of the sense organ to come into contact with a pheromone present on the female. (iii) Mating takes place in the dark and visual cues are not necessary; success in mating could be due to chemical cues and or vibrational cues. (iv) Male's approach to a female *Limnoporus dissortis* is different, and, once contact is made, the male retreats (vibrational cues cannot be considered because females of both species do not respond to signalling).

Evidence that does not support a pheromone-mediated mating system is from findings in behavioural experiments: (i) Experiments in which the receptors are blocked by paint demonstrate that males are able to mate with non-functioning receptors. (ii) Experiments where models are treated with extract of slurreyed females and presented to males show no receptiveness to males concerning mating.

It is difficult to state strong support for a pheromone-mediated mating system; at the same time, it is equally difficult to falsify the hypothesis that this type of system exists. The fact that nymphs and females do not have the sensilla is very strong evidence of function in mating behaviour. The leg rubbing act is strong evidence of a contact pheromone related behaviour. Behaviour of males with *Limnoporus dissortis* females demonstrate that cues other than vibrational and visual play a rôle in species recognition. The ability to mate in the dark is not particularly supportive of a pheromone-mediated mating system, but it is consistent with the hypothesis.

Results from experiments where the receptors were blocked are the strongest evidence against a pheromone-mediated mating system. It shows that the rôle of these sense organs is — at best — an enhancement to successful mating. The inability of these males to approach and orient (when contact is made) in the usual manner could be a result of improper input from the sense organs; it may also be due to general malaise of the insects because of presence of paint.

Considering findings from all aspects of this study, I think that the chemosensitive basiconic sensilla in *Gerris remigis* serve some function in mating behaviour (regardless of their function in the *Limnoporus rufoscutellatus* group). However, I cannot conclude, with any certainty, that the function is that of detecting a contact pheromone. I believe that the rôle of these sense organs is but a part of a larger mating system in which visual, vibrational, and chemical cues all play a part in the mating behaviour strategy of *Gerris remigis*. I believe all three cues (and their detection) are important, but not all three cues need be present for successful mating to occur.

Through this study, cuticular structures of the legs have been discussed. A description of a previously undescribed sense organ has been made. Its distribution within the family Gerridae has been surveyed. And, its physiological function has been determined. In an attempt to elucidate the behavioural function of this sense organ, a complete mating sequence of *Gerris remigis* has been documented.

Future studies should entail more histology of the sense organ at the TEM level. More behavioural experiments should be carried-out, paying close attention to extraction of putative pheromones. And, electrophysiological studies should be undertaken, especially if behavioural experiments prove promising.